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|---|---|---|---|---|---|---|---|---|---|
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# THE OCCURRENCE OF CITRIC ACID IN URINE

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*From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago*

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With the intention of studying the fate of citric acid introduced in the organism by various routes and in various diseases, we tested the applicability of the method of Kunz to the quantitative determination of citric acid in urine and other body fluids. Briefly, the procedure of Kunz (1) is as follows: Evaporated wine is brought to a volume of 100 cc. under addition of 2 cc. dilute sulphuric acid and 20 cc. bromine water. To 50 cc. of the clear filtrate 10 cc. 1:1 $H_2SO_4$ and 3 cc. 37.5 per cent KBr solution are added. The flask is kept five minutes in a water bath of 50°. After removing from the bath 25 cc. of a 5 per cent $KMnO_4$ solution is run in from a pipette under vigorous shaking, not permitting the temperature during oxidation to rise above 55°. Finally, the remaining undissolved $Mn(OH)_4$ and the excess of bromine are removed by the addition of concentrated $FeSO_4$ acidified with $H_2SO_4$. Kunz then brings the precipitated pentabromacetone on a tared filter paper; washes well and dries in vacuo over $H_2SO_4$. He gives as the limit of his method 0.02 per cent citric acid ($C_6H_8O_7$ + 1 $H_2O$) that is 0.01 gram for the actual determination. We adhered to the procedure of Kunz inasmuch as the volume of the fluid before the addition of the strong $H_2SO_4$ and of the KBr was always 50 cc. We replaced the tared filter paper by Gooch crucibles, because filter paper blanks always lost from 2 to 4 mgm. weight and we dried the precipitate without the use of the vacuum, because it loses weight appreciably in vacuo. In a series of determinations the crucibles were left in the desiccator twenty-four hours and weighed. After another twenty-

four hours in the desiccator, there was a loss of weight from 0.1 to 0.3 mgm. (the greatest loss in a number of such experiments was 0.5 mgm.). The crucibles were then returned to the desiccator which was now exhausted with the vacuum pump for one hour. After standing in vacuo for twenty-four hours, the loss was from 2.4 to 4.1 mgm. Standing in air at room temperature, pentabromacetone loses weight very gradually. This behavior of pentabromacetone precludes an absolutely accurate determination. We weighed the precipitates after being left in the desiccator twenty-four to forty-eight hours. The weight of the pentabromacetone $\times$ 0.464 gives the weight of citric acid + 1 $H_2O$.

In our first experiments, to which we will refer here very briefly, 200 to 250 cc. of urine, to which sodium citrate had been added, was evaporated, taken up with a little water and 80 per cent $H_3PO_4$, mixed with plaster of paris and extracted with ether at least eighteen hours. The ether was driven off, the residue taken up in water, boiled with a little animal charcoal and filtered. The clear filtrate was used for the determination. In this way relatively little of the added citric acid could be determined, much more when the urine had been diluted with water and exposed to the action of urease. This increase in the yield could not be accounted for by the amount of pentabromacetone obtained from urease alone.

Extracts of Arlco-urease as well as of powdered soy and jack bean yield pentabromacetone on similar treatment, and the final solutions obtained from the ether residue give a positive Denigès reaction.

It was found that the pentabromacetone reaction could be carried out with native urine. Before giving the data of some of our experiments, we record in table 1 results obtained with an aqueous solution of sodium citrate of known strength. In the first column, the amount of citric acid + 1 $H_2O$ used for the determination is given in milligrams; in the second, the amount found in milligrams and in the third, the difference between these two values.

With 5 mgm. of citric acid sometimes none or a hardly perceptible turbidity occurs, only to be recognized when compared with a blank, sometimes this turbidity is more marked and yields a trace of a precipitate. That is, the visible limit of the reaction is reached with about 5 mgm. citric acid. With larger amounts there remains an undetermined rest shown in the last column of the table and this undetermined rest seems to be rather constant and may perhaps become more constant with improvements in the method. The average of the figures for the

undetermined rest is 5.5 mgm., very near the visible limit of the reaction. Naturally, whenever the Kunz method of the determination of citric acid is used in the manner indicated, this undetermined rest must be taken into consideration.

Dry sodium citrate was added to filtered normal urine in amounts corresponding to those of citric acid given in table 2. Each test was made with 20 cc. urine, to which 1 cc. dilute $H_2SO_4$ and 10 cc. bromine water was added, bringing each specimen up to 50 cc. with water. *A*

TABLE 1

| CITRIC ACID + 1 $H_2O$ | CITRIC ACID FOUND | DIFFERENCE |
|---|---|---|
| *mgm.* | *mgm.* | *mgm.* |
| 10 | 5.2 | 4.8 |
| | 5.0 | 5.0 |
| 15 | 9.8 | 5.2 |
| | 9.5 | 5.5 |
| 20 | 14.9 | 5.1 |
| | 14.5 | 5.5 |
| 25 | 19.3 | 5.7 |
| | 19.1 | 5.9 |
| 30 | 24.3 | 5.7 |
| | 25.0 | 5.0 |
| | 24.1 | 5.9 |
| | 24.4 | 5.6 |
| 40 | 34.3 | 5.7 |
| | 33.7 | 6.3 |

and *B* signify different sets of experiments. The controls of series *A* with urine alone showed a slight turbidity; those of *B* more, and some gave a little precipitate with the characteristic odor of pentabromacetone. In both series the controls acted like aqueous solutions of sodium citrate, containing just about the limit of demonstrable citric acid.

For this reason we did not make the correction for the undetermined rest. With amounts greater than 10 mgm. citric acid, it is seen that the pentabromacetone method gives quite satisfactory approximate results. At first we did not feel quite sure about the character of the precipitate given now and then by 20 to 30 cc. fresh urine in spite of the

odor of pentabromacetone. But when we tried to determine added citric acid in larger amounts, such as 100 to 300 cc. by evaporating the

TABLE 2

| | CITRIC ACID + 1 $H_2O$ MGM. | | PER CENT RECOVERY |
|---|---|---|---|
| | Found | Added | |
| | 49.0 | 50 | 98 |
| | 49.1 | | |
| | 29.2 | 30 | 98 |
| | 29.3 | | |
| | 18.9 | 20 | 95 |
| | 19.1 | | |
| *A*..... | 8.3 | 10 | 86 |
| | 8.9 | | |
| | 3.5 | 5 | |
| | 5.7? | | |
| | 1.6 | 2.5 | 60 |
| | 1.4 | | |
| | 47.2 | 49.6 | 97 |
| | 48.9 | | |
| | 28.6 | 30 | 96 |
| | 28.5 | | |
| | 19.5 | 20 | 98 |
| | 19.8 | | |
| *B*..... | 9.0 | 10 | 94 |
| | 9.8 | | |
| | 3.8 | 5 | 81 |
| | 4.3 | | |
| | 1.9 | 2.5 | |
| | 2.6 | | |

urine and making up the filtrate from the residue to 50 cc., etc., we found that every normal urine, that of infants included, yielded a pre-

cipitate which could be identified as pentabromacetone, by its odor, its melting point of 73°, by the precipitation from its solution in acetone, alcohol and ether with water and by the fact that it could be distilled in a current of steam.

The question of the derivation of this pentabromacetone now arose. There are not many substances known to yield pentabromacetone easily. Meyer and Jacobson (2) mention besides citric and aceton dicarbonic acid, $\gamma$ pyron and chelidonic acid. In all these cases, the pentabromacetone is finally derived from aceton dicarbonic acid.[1] Kunz reports satisfactory results with synthetic solutions containing tartaric, succinic, malic and lactic acid and glycerol, that is, none of these substances interfere with the reaction.

We tested among other substances, succinic, lactic, uric acid, l. $\beta$ hydroxybutyric acid[2] separately and all with negative results. Neither did glycerol nor glucose nor a specimen of pyruvic acid (Kahlbaum) give any reaction. The pyruvic acid has been in the laboratory several years and such specimens are known to contain condensation products.

We had a specimen of campho glycuronic acid at our disposal prepared according to Schmiedeberg and Meyer (3) from the urine of rabbits injected with camphor dissolved in oil. The reaction was negative.

Tricarballylic, aconitic, ita, citra and mesaconic acid, more or less closely related to citric acid, did not yield any pentabromacetone. Peculiar results were obtained with two specimens of urine containing diacetic acid. One came from a diabetic patient, the other from a case of so-called cyclic vomiting. Of the diabetic urine 25 cc. boiled shortly with the addition of 1 cc. dilute $H_2SO_4$ did not give any reaction, while the unboiled urine gave a positive reaction.

The unboiled specimen of the urine from the second case gave a more pronounced reaction than the boiled. Diacetic acid, freshly prepared from its ethyl ester, was added to normal urine, and in some instances glucose was added also. The reaction seemed to be more pronounced when diacetic acid was added and in one series, where the controls did not give any reaction, the specimens with added diacetic acid gave a definite reaction with a little precipitate. In another series the controls gave a little precipitate and the specimens with the added diacetic acid seemed to show more turbidity and more precipitate, but there was only very little difference in the weight. A solution of

[1] Beilstein states it has been obtained from pyrotartaric acid with a melting point of 76°.

[2] We owe this acid to the kindness of Professor Shaffer.

diacetic acid shows a little peculiarity in the course of the reaction. On careful addition of the $KMnO_4$ there occurs a turbidity somewhat different from that seen in the usual reaction and on clearing the mixture with $FeSO_4$ none of the turbidity remains, in contrast to the behavior of citric acid solutions, where such a turbidity on addition of $KMnO_4$ is a sure indication of a positive reaction. Solutions of diacetic acid do not give a pentabromacetone reaction. Its presence in urine seems to have some influence on the reaction under certain hitherto unknown conditions.

The evidence is not absolutely conclusive that the pentabromacetone obtained from urine under the conditions of the experiment is derived from citric acid.

At the same time, until proof to the contrary is brought, we are fully justified in accepting citric acid as its source and therefore to claim citric acid as a constituent of normal human urine.

About this time Salant and Wise (4) published their method for the quantitative determination of citric acid based on the Denigès reaction. A number of urines from normal individuals and from patients were tested qualitatively in both ways and with exception of one gave both reactions. This urine from an atrophic infant gave a negative pentabromacetone reaction with over 200 cc. and it required the equivalent of 30 cc. urine to give a slight but definite Denigès reaction, when carried out according to Salant and Wise, the reaction being negative or doubtful with less. The positive Denigès reaction would be much stronger corroborative evidence for the presence of citric acid if it were not, like the pentabromacetone reaction, due to the production of aceton dicarbonic acid. Nevertheless, when we tested some of the substances mentioned above, substances giving a negative pentabromacetone reaction, we obtained positive Denigès reactions. These substances were pyruvic, or perhaps condensation products contained in our specimen, aconitic, ita- and citraconic acid.

It is of interest to note that neither tricarballylic nor mesaconic acid gave a reaction. The tricarballylic acid does not react at all on addition of the permanganate solution, the solution becoming and staying red after addition of the first drop. The itaconic acid was obtained as residue from a mixture of ita- and citraconic acid on distillation in a current of steam. Its melting point was 161° (uncorrected). The citraconic acid used for the tests was the oily part of the dry distillation of citric acid. The substance crystallized on evaporation, the crystals being of fatty appearance. Its melting point was not sharp, beginning

at 84°. The mesaconic acid was prepared by boiling a mixture of ita- and citraconic acid in 10 per cent NaOH for six hours. The melting point was about 200° (uncorrected.)[3] There are some differences in the course of the reaction when compared with the reaction of a citrate solution. It seems to require larger amounts particularly of aconitic acid to get a positive reaction. Furthermore, in the case of citrate solutions, the turbidity can be definitely seen, or when more concentrated, a precipitate is formed long before the color changes on addition of the $KMnO_4$. With the substances mentioned above, the precipitate becomes visible and seems to be formed on clearing with $H_2O_2$. Whether this precipitate is identical with that obtained from citric acid remains to be seen. It is doubtful whether the reactions just described detract from the value of the Salant and Wise method for the quantitative determination of citric acid in urine. But the fact that urine properly treated gives a positive pentabromacetone and a typical Denigès reaction, in the light of such occurrences, points very strongly to citric acid as the substance responsible for these reactions.

Taking into consideration the occurrence of citric acid in milk, it is not at all strange that it should also occur in urine. From the feeding experiments of Salant and Wise and from some of our observations on patients receiving large doses of sodium citrate per os, it is very improbable that the citric acid of the urine should come from citric acid contained in food. Dr. Rettinger, a pupil of Marchlewski, called our attention to a very interesting possibility, that is, the derivation of citric acid from hemopyrrol over an hematinic acid from which a partial anhydride of ethyl aconitic acid and ethyl tricarballylic acid have been obtained (5). This possibility would naturally include that of the derivation of citric acid in plants from chlorophyl.

Having established rather firmly the presence of citric acid in normal urine of infants, children and adults, it will be necessary to determine the amount excreted in a unit of time and its variations under various conditions. Some of this work has been started. Since 20 cc. of urine do not contain as a rule a sufficient quantity of citric acid to permit the use of the pentabromacetone method for its approximate quantitative determination, more urine can be taken. With normal urines, as much as 50 cc. filtered urine can be used under the arrangement of the experi-

[3] Citric acid heated with concentrated $H_2SO_4$ in a current of $CO_2$ gives CO, molecule per molecule. Tricarballylic, aconitic, ita—citra—and mesaconic acid all yield CO under such conditions, but not molecule per molecule, and the three last ones yield about the same amount, and more than the first two.

ments to which we adhered. To this 0.5 to 1.0 cc. $H_2SO_4$ may be added and bromine vapor dropped in. If no precipitate is formed, and thus far this never happened with normal urine, the pentabromacetone reaction can be carried out immediately. If the urine contains more than 10 mgm. citric acid rather good results may be obtained when compared with the results obtained by the method of Salant and Wise.

We will give a few examples later on. If the urine does not contain sufficient citric acid a larger amount may be evaporated with the addition of dilute $H_2SO_4$. The residue is taken up in water and the clear filtrate used for the determination. These solutions are sometimes very highly colored. To avoid this, we boiled the urine after the addition of dilute $H_2SO_4$ with animal charcoal. This procedure recommended itself furthermore for pathologic urines. The results obtained in some of the earlier experiments with the addition of citric acid to urine seemed to permit this process. Hereby a certain quantity of citric acid was added to urine, then some dilute $H_2SO_4$ and animal charcoal (Blutkohle Kahlbaum), the urine was boiled and filtered and of the filtrate a given amount was used. The residue on the filter was not washed. Of a sample containing 60 mgm. citric acid, 56.5 mgm. were determined and again another time 58.8 mgm. were added to urine, while 60.5 mgm. were found, here the control was negative, that is, it contained less than 5 mgm. citric acid.

In a series of experiments arranged as in table 2 the percentage recovery on addition of

| | |
|---|---|
| 50 mgm. citric acid was | 99 |
| 30 mgm. citric acid was | 95 |
| 20 mgm. citric acid was | 88 |
| 10 mgm. citric acid was | 77 |

Such results prompted us to employ animal charcoal in a number of experiments by preference. Later we found that sometimes the treatment with animal charcoal may entail considerable losses. Aqueous solutions of sodium citrate lose an appreciable amount of citric acid when treated with animal charcoal; for instance, from 30 mgm. citric acid, 21.7 mgm. and from 49.4 mgm. citric acid 41.1 mgm. were found, including the correction for the undetermined rest.

It seems that charcoal does not always adsorb citric acid from urine with the same avidity as from aqueous solutions.

The following table 3 gives the results of experiments as indicated. The figures represent milligrams citric acid found per 100 cc. urine. The

specimens of urine were mixtures obtained from several individuals. The first horizontal column gives the results obtained with 50 cc. filtered urine; the second, the results obtained with either 100 or 150 cc. urine after evaporation; in the third column, 250 to 350 cc. of urine were made strongly acid with sulphuric acid, animal charcoal was added and the urine boiled and filtered; of the filtrate 100 to 150 cc. were evap-

TABLE 3

*Citric acid per 100 cc. in milligrams*

| | | | | | |
|---|---|---|---|---|---|
| (1) | 50 cc. urine direct | | 20.5 | 21.2 | 29.1 |
| (2) | 100 to 150 cc. evaporated. No animal charcoal. | 25.6 | 19.0 | 20.9 | 21.5 |
| (3) | 100 to 150 cc. filtrate from urine treated with animal charcoal | 25.2 | 9.0? | 21.0 | 25.5 |
| (4) | Filtrate from 100 to 150 cc. urine treated with charcoal residue washed | | 15.7 | 17.2 | 21.0 |
| (5) | Salant and Wise | 31.3 | 22.2 | | 29.5 |

orated, etc. In the fourth column 100 or 150 cc. of urine were heated with animal charcoal and filtered. The residue on the filter was taken up in boiling water; filtered and thoroughly washed with hot water. The washwater was added to the first filtrate, the total evaporated, etc. The fifth horizontal column contains the figures obtained by the method of Salant and Wise.

The results seem to indicate that the best results with the pentabromacetone method may be obtained using the urine without any further treatment, where that is possible. In how far the pentabromacetone method can be utilized for the quantitative determination of citric acid in urine has to be left to further work. While more time elapses to obtain the results, the method has the advantage over that of Salant and Wise in that it requires fewer and simpler manipulations.

In table 4 we give the results obtained with twenty-four hour specimens of urine from three normal adults (A., G. and R.). Urine was

TABLE 4

*Citric acid in milligrams*

| | PENTABROMACETONE METHOD (ANIMAL CHARCOAL) | | SALANT AND WISE | |
|---|---|---|---|---|
| | Per 100 cc. | Per twenty-four hours | Per 100 cc. | Per twenty-four hours |
| R. | 47.0 | 475 | 50 | 505 |
| G. | 35.2 | 440 | 43 | 536 |
| A. | 21.8 | 440 | 27 | 548 |

taken for the determination by the method of Salant and Wise. The rest was boiled with animal charcoal and filtered. In the first two instances 50 cc. of the cooled filtrate served for the determination, in the last 300 cc. were evaporated.

It is seen that quite an appreciable amount of citric acid is excreted in the course of twenty-four hours, enough to play a modest rôle in the acid-base economy of the organism.

## CONCLUSION

Normal human urine contains citric acid.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 298.

# THE RECEPTION OF MECHANICAL STIMULI BY THE SKIN, LATERAL-LINE ORGANS AND EARS IN FISHES, ESPECIALLY IN AMIURUS

GEORGE HOWARD PARKER AND ANNE P. VAN HEUSEN

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## INTRODUCTION

Students of the sense organs of the lower vertebrates are coming to recognize more and more generally the connection between the organs of touch in the skin, the sense organs of the lateral-line system and the sensory patches of the internal ear as a group of closely related receptors. The intimate connections of these sets of organs are not only indicated in their structural and functional similarities but also in their central relations, as shown by Strong, Johnston, Herrick, Cole, and more recently by Kappers ('09), conditions which justify their classification under a common head, the somatic afferent system. It is therefore quite obvious that a consideration of the physiology of one of these sets of receptors cannot well be carried out without at the same time dealing with that of the others. Hence we have undertaken a study of the skin, lateral-line organs and ears in fishes, especially in Amiurus.

Such an investigation involves two important and vexed problems, the function of the lateral-line organs and the question of hearing in fishes. Both of these are discussed in the present paper and it is hoped that the new facts brought forward will help to clarify these somewhat obscure topics.

## MATERIAL AND TECHNIQUE

Sumner ('07, p. 79), in his study of the relations of fishes to the physical and chemical features of their environment, calls attention to the fact that the catfish, Amiurus, when slightly poisoned with strychnine is very responsive to noises, and Maier ('09, p. 394), who reports his inability to obtain any response to sound from a considerable range of marine and fresh-water fishes, records with more or less surprise one instance of reaction to this stimulus in the common American catfish,

Amiurus nebulosus. This fish is said to retreat to cover very generally when a shrill whistle is blown.

As Amiurus nebulosus is very abundant in the neighborhood of Cambridge and is apparently very responsive to mechanical stimuli, we decided to test it out fully for the skin, the lateral-line organs and the ears, but we have made no attempt to work on the possible functional relations of the ears in this fish to the system of Weberian ossicles and the swim bladder.

Amiurus has many advantages for experimental work. As Lavanden ('05) observed, it is extremely hardy, it will live for a long time out of water and it recovers easily and quickly from operations. The chief difficulty with it is that it is very subject to attacks of saprolegnia in the spring and at this time of year, because of the high death rate, it is often impossible to keep a stock of these fishes in aquaria. In the early stages of an attack the animals can sometimes be cured by an exposure to sunlight or by treating the skin with a strong solution of sodium chloride, after which natural healing sets in and a recovery may be accomplished. Such a solution is also to be recommended for disinfecting aquaria and other such vessels.

Amiurus nebulosus is apparently a gregarious fish. At least a single individual in a large aquarium swims incessantly and restlessly about and never becomes quietly responsive till it is with others of its kind. Under the latter circumstances it often rests on the bottom of the aquarium or floats lazily in mid-water or near the top, and in this state of quiescence it will exhibit reactions not seen under any other conditions. We, therefore, were obliged to give up attempting to work with these fishes singly and always dealt with them in pairs or in groups of larger numbers. A pair of fishes seemed to be in all respects as quiescent and responsive as larger assemblies were.

Another peculiarity of Amiurus is that it is active chiefly at night. During the day it remains relatively quiet and usually withdraws to as secluded a position in the aquarium as it can find. It is therefore evident that a fish in the dark is in a more active and responsive state than one in the light. For this reason blinded fishes, as we found by experiment, swim almost continuously. For our experiments, however, it was desirable to have the fishes in as nearly normal a state as possible and we therfore did not operate and blind them, but devised a temporary cover for their eyes. This was made of very thin kid and had the form of a pair of goggles (fig. 1). These were sewed at two points to the skin of the fish's head so as to cover the eyes. Thus without serious

operation the fishes were effectually blindfolded, though it is to be remembered that they are also responsive to light through the skin (van Heusen, '17). Such blinders may remain on a fish for as much as two weeks and seemed to disturb it little, if any, except in so far as they deprived it of sight.

Experimentalists in the past have found it very difficult to eliminate the three sets of sense organs with which we planned to work without seriously disturbing the fish. Nor can we say that we have attained ideal results in this respect. But we have worked with a variety of methods and believe that those that we finally adopted are decidedly superior to the means formerly employed.

Fig. 1. Dorsal view of a blindfolded Amiurus, showing the blinders stitched to the fish's skin and covering the eyes.

To eliminate the skin as a receptive surface extensive nerve cutting has heretofore been employed (Parker, '03 a; '03 b; Bigelow, '04). The severance of the root of the trigeminal complex on each side of the head and of the spinal cord just behind the pectoral fins eliminates most of the integument, but, as Körner ('05, p. 113) has pointed out, this leaves the skin of the pectoral fin region still sensitive and the operation, as any one who has tried it knows, reduces greatly the capacity of the fish for muscular response.

We therefore endeavored to devise a wholly new way of desensitizing the skin. Local anesthesia seemed a promising means and for this purpose we employed dilute solutions of the following substances: alcohol, ether, chloroform, chloretone, stovaine, novocaine, cocaine, urethane and magnesium sulphate. These were applied to the skin of the fish, their effects studied and recovery watched. None proved so satisfactory as an aqueous solution of magnesium sulphate. After trying various strengths of this solution for different times, we decided that a 20 per cent solution applied for five minutes was the best combination

to use. At first we applied this solution rather freely to the fish held in a wet cloth, but much of the salt entered the mouth and gill openings and produced marked internal disturbances. We finally devised the plan of holding the fish vertically in air by a narrow band of cloth around its body just posterior to the pectoral fins and then painting the skin with the solution of magnesium. In this way very little if any of the salt entered the mouth or gills. On returning a fish to the aquarium after five minutes' treatment of this kind, the animal begins in a few minutes to swim quietly about. Its locomotion is somewhat labored and its equilibrium a little disturbed, but otherwise its actions are essentially normal. The skin of such fishes remains insensitive to touch for an hour to an hour and a half after the application of the salt. This can be ascertained by touching it from time to time with a rod. After an hour and a half sensitivity gradually returns. Amiurus may be subjected to many such treatments in succession, but it is well not to repeat the process too frequently, especially in the spring, for the outermost layer of the skin is often killed by the salt and sloughed and the animal is thus rendered more open to attacks of saprolegnia than untreated fishes are. Notwithstanding this drawback, desensitization of the skin by magnesium sulphate is a vast improvement over nerve cutting and so far as our experience goes, it is the best method by which to obtain the desired end.

The elimination of the lateral-line organs has been attempted in a variety of ways. Richard ('96) removed scales from the region of the lateral-line and then burned out the sense organs with heat, an operation that commonly resulted in the death of the fish. Bonnier ('96) and Lee ('98) used electro-cautery and thermo-cautery to destroy the receptors. Parker ('05) eliminated the sense organs by cutting nerves in appropriate positions. Hofer ('08) cut the lateral-line nerve near the pectoral girdle and burned out the sense organs on the head. All these methods are severe; they usually involve a considerable loss of tissue and fishes seldom live long after such treatment. We therefore attempted to improve these operations. The lateral-line organs of the trunk were eliminated by cutting the lateral-line nerve near the gill clefts. The nerve is very superficial and is easily reached at this point; the operation is always successful and the slight wound heals readily. The distribution of the lateral-line organs on the head of Amiurus has been most carefully worked out by Herrick ('01), whose account we have confirmed in many particulars by dissection. In Amiurus there are twenty-four of these organs on each side of the head and their locations

are easily made out by reference to the external pores. These sense organs were destroyed individually by a depilating needle. Six Columbia dry-cells were set up in parallel. To the positive pole of this battery was attached a broad metal plate on which the fish was placed. To the negative pole was fixed a depilating needle such as is used by dermatologists for the removal of superfluous hair. This needle was inserted for thirty seconds into the spot where a sense organ was believed to be. The point of insertion in the skin quickly became surrounded by a dark ring and from the slight wound a yellowish froth oozed. It was customary to destroy the sense organs of only one side of the head at a time, reserving those of the other side for subsequent treatment. Several weeks after the completion of such an operation, sections of the tissue showed the sense organs to be fully degenerated. The fishes survived the operation many months, one in fact lived over a year, and we therefore feel certain that though the lateral-line organs were completely destroyed, the general activities and length of life of the fishes were not seriously impaired.

The elimination of the internal ear has been attempted by its removal, as practised by Kreidl ('95), by injecting cocaine, as was done by Gaglio ('02) and by von Marikovszky ('03), and by cutting the eighth nerve, an operation employed by Parker ('03 a) and by Bigelow ('04). The defectiveness of Kreidl's method has already been pointed out by Bigelow ('04) and the injection of anesthetics has never given good results in our hands. We have, therefore, resorted to the former method (Parker, '03 a) of cutting the eighth nerve, which can be accomplished in Amiurus through a very small incision that quickly heals. The cutting of this nerve is almost invariably followed by loss of equilibrium and general muscular weakness, but we have felt it necessary to dissect after death all experimental animals to insure that the nerve had been cut, for the eighth nerve, unlike the lateral-line nerve, cannot be seen in the operation of cutting it.

By combining the methods for the elimination of the lateral-line organs and the ear we have obtained fishes without either of these receptors. The anesthetizing of the skin with magnesium sulphate is necessarily a more temporary measure, but this nevertheless can be depended upon for at least an hour. By combining these various methods we have been able to obtain fishes giving all possible combinations of the three sets of receptors with which we planned to work. Including normal individuals and those without any of these three sets of organs there are eight such classes of fishes.

1. Normal fishes with skin, lateral-line organs and ears effective swim in a leisurely way up and down the midst of the water. Often, however, they come to the surface and swim with the mouth open, the upper jaw being held slightly above the level of the water. Since the stomach of Amiurus often contains aquatic insects whose habit it is to swim or float on the surface of the water, it seemed probable that this fish is not entirely a bottom-feeder but resorts to the surface and feeds there. A second object in coming to the surface is to snap air as a respiratory adjunct. Fishes have been observed to remain in a vertical position, snapping air in such a way as to form large quantities of air bubbles around their mouths. Normal fishes spend about equal amounts of time in swimming and in resting. When Amiurus comes to rest it seldom lies flat on the bottom. Usually it hangs against the end of the aquarium, giving the appearance of standing on its tail. The dark part of the aquarium was most often sought by Amiurus as a resting place, showing thus a negative reaction to light, in which the skin was probably the receptor.

2. Fishes in which the skin and ears are intact but from which the lateral-line organs had been eliminated, swim more rapidly than normal fishes and with somewhat jerky movements. They make sudden darts and jumps on slight provocation and on the whole show what might be called a nervous disposition. They spend much more time swimming than normal fishes do.

3. Fishes that retain the skin and the lateral-line organs in normal condition but in which the ears are no longer effective are in many ways just the reverse of those in group 2. Most of their time is spent lying on the bottom of the aquarium often ventral side up in consequence of disturbed equilibrium. When they swim it is usually in a slow rolling way or if they are driven to rapid locomotion, they move in grotesque spirals. Some stimuli set up in these fishes a series of backward jerks, which are repeated many times. These movements were not noticed in fishes in which the ear was intact.

4. If fishes in which the ear had been rendered ineffective were now operated upon for the lateral-line organs, leaving only the skin intact, group 4, there followed an increase in activity and much of the sluggishness characteristic of fishes in which only the ears had been rendered ineffective disappeared. This renewed activity was not far from that of a normal Amiurus.

The remaining groups, 5 to 8 inclusive, repeat the conditions in groups 1 to 4 except that in groups 5 to 8 the skin is always insensitive. In

their reactions these fishes also resemble those of the first four groups except that in each group they are a little more sluggish than those of the corresponding group among the first four. Thus fishes in group 5, in which the skin is anesthetized but the lateral-line organs and ears remain intact, resemble normal fishes, group 1, except that they are not quite so active. Those of group 6, in which the skin and lateral-line organs are eliminated but the ears are undisturbed are like less active members of group 2. In group 7, in which the lateral-line organs are the only effective receptors left and in group 8, in which all three sets of sense organs have been eliminated, locomotion is rare though more frequent among members of group 8 than of group 7. All fishes in which the skin has been anesthetized swim in a somewhat labored way and with slightly disturbed equilibrium.

These eight groups of fishes were all subjected to each of the various forms of mechanical stimuli employed by us and the records of their responses are contained in the following pages.

## RESPONSES TO MECHANICAL STIMULI OTHER THAN SIMPLE SOUNDS

In experimenting on Amiurus with mechanical stimuli, we used a large laboratory aquarium that measured on the inside 75 cm. in length, 35 cm. in breadth and 40 cm. in height (fig. 2). Amiurus, as the observations of others have already shown, is extremely responsive to mechanical disturbance. We therefore mounted the aquarium so as to exclude all possible causes of disturbance. The work was carried on in a basement room the floor of which was built directly on the earth. The aquarium (fig. 2) stood on a firm table, each leg of which was supported as follows: next the concrete floor was a plate of rubber 20 cm. by 20 cm. and 1.8 cm. thick; on this rested a tile of the same size as the rubber and 2.5 cm. thick; on the tile was a mass of "excelsior" wood shavings, on which the leg of the table rested. On the table top was an inflated bicycle tire which carried a light wooden platform on which the aquarium rested. By these devices we believe we excluded all significant vibrations from the earth and our various tests supported this opinion. The laboratory by good fortune is far from railroads and electric carlines.

The bottom and ends of the aquarium were made of heavy slate and the sides were of plate glass. Inside on the bottom of the aquarium was spread a thick sheeting padded with cotton batting, as a means of eliminating mechanical disturbances that might make their way into

the aquarium when, for instance, it was moved on its own base. To restrain a little the fishes to be experimented upon, an enclosure made of coarse cheese-cloth and measuring about 35 cm. square and about 10 cm. deep, was often used. It was hung at the surface of the water and next the end of the aquarium at which the stimulus was delivered. The aquarium was placed so as to receive light from a north window, but it was ordinarily illuminated by an overhead electric light.

In stimulating the fish a variety of mechanical contrivances was used. A leaden ball weighing 335 grams was suspended from the ceiling by a wire 118 cm. long and was used as a pendulum to strike against one of the slate ends of the aquarium. This end was 3.1 cm. thick. At rest the ball was just in contact with the aquarium and the blow was usually given by letting it swing through 5 degrees of arc whereby it attained a calculated velocity of 29.7 cm. per second. It was allowed to strike the aquarium wall once and was caught on the rebound.

Fig.2. Aquarium in which experiments on Amiurus were conducted; the aquarium is mounted so as to prevent mechanical disturbances from reaching it through its support; in the upper part of the left-hand end is the small cage in which the fishes to be tested were placed; outside the left-hand end and entirely separated from the aquarium and its support except for the connecting electric wires is a base carrying the switch by which the observer could make or break the current from the current generator to the telephone submerged in the water in the small cage.

A second form of stimulus was of a slow vibratory kind produced by pressing on the aquarium on one side so as to deform slightly the inflated bicycle tire on which it rested and then, on suddenly releasing it, to let it rock slightly back and forth. This movement was perceptible through waves produced by it on the surface of the water in the aquarium. When a kymograph record of the vibratory movements of the aquarium as a whole was taken, it was found to begin approximately at 4 to 5 complete swings per second

after which the waves on the surface of the water continued at the rate of 16 in ten seconds or 1.6 per second. These low vibratory movements are necessarily not open to very accurate control.

A third form of stimulus was a local current produced by leading water from an overhead reservoir into the aquarium through a small glass tube. The flow was carried out under a pressure of 50 cm. of water and the aperture at the end of the glass tube through which the current escaped had a diameter of 0.7 mm.

A fourth form of stimulus was obtained by allowing one or more drops of water to fall through a distance of 5 cm. and strike the surface of the water in the aquarium.

A fifth stimulus was the sound produced in air by a shrill watchman's whistle blown vigorously.

All these stimuli have much in common and may be considered more or less together. Each stimulus was applied to several, often to many representatives of the fishes already described. Fishes of the appropriate type to be tested were blindfolded and allowed to remain there some hours or even days until they were thoroughly at home. When this condition had been arrived at, the particular stimulus to be tried out was applied at regular intervals and a record of the reactions was taken.

As there were five kinds of stimuli and eight classes of fishes to be tested, it required a long time to complete the records; in fact this part of the work extended over a period of more than two years. In some classes of responses many more observations were needed and taken than in others and consequently the final series of records is based upon different numbers of observations in different cases. It seemed best, therefore, in tabulating these results to express them in percentages rather than in absolute numbers; comparisons would be facilitated thereby. A condensed statement of the results in percentages is given in table 1. It should be remarked that in no instance does any figure in the table represent an average of less than ten records and in most instances the number of separate records is much higher.

A discussion of the contents of table 1 can best be begun with the responses to the whistle. If the fish is well below the surface of the water in the aquarium and the whistle is blown, there is as a rule no response whatever. The records on which the tabulation under "whistle in air" is based were taken from fishes that were directly on the surface of the water when the whistle was blown. The response is a quick turning and swimming downward into the deeper water. The

response is extremely constant in occurrence, when it occurs at all, and is characteristic of fishes in groups 1, 2, 5 and 6 of the table. It is as characteristically absent from groups 3, 4, 7 and 8. When the conditions of these groups are noted, it is clear that the response occurs in all fishes in which the ears are intact and is absent from all those in which the eighth nerves have been cut. It would be difficult to find a clearer case of auditory response than this group offers.

The response to the drops of water on the surface of the aquarium is also a simple form of reaction. Here, too, the response is a quick retreat on the part of the fish, to be noted only when the animal is near

TABLE 1

*Responses, expressed in per cents of the total number of trials, of fishes representing the eight combinations of receptors to various forms of mechanical stimuli*

| SENSE ORGANS EFFECTIVE IN EACH OF THE EIGHT CLASSES OF FISHES | MECHANICAL STIMULI | | | | | |
|---|---|---|---|---|---|---|
| | Whistle in air | Drops of water | Current of water | Vibration of whole aquarium | | Blow from lead ball |
| | | | | Fish near surface | Fish in mid-water | |
| 1. Skin, lateral-line organs and ear | 100 | 100 | 100 | 100 | 88 | 96 |
| 2. Skin and ear | 100 | 100 | 100 | 98 | 90 | 93 |
| 3. Skin and lateral-line organs | 0 | 100 | 87 | 100 | 40 | 11 |
| 4. Skin | 0 | 100 | 88 | 97 | 27 | 27 |
| 5. Lateral-line organs and ear | 100 | 0 | 0 | 80 | 22 | 33 |
| 6. Ear | 100 | 0 | 0 | 94 | 26 | 73 |
| 7. Lateral-line organs | 0 | 0 | 0 | 0 | 0 | 0 |
| 8. None | 0 | 0 | 0 | 0 | 0 | 0 |

the surface though not close enough to the drop to be hit by it as it descends into the aquarium water. Groups 1 to 4 inclusive invariably responded to this stimulus and groups 5 to 8 inclusive with equal completeness failed to respond. Here also the relation to the receptor is simple and direct. All those fishes in which the skin is intact respond to the dropping of water and all others fail to respond. Obviously the receptors in this instance are integumentary organs and without doubt the tactile organs of the skin.

Very similar to the response to a drop of water is that to a local current. This current which, as stated, emerged through an outlet 0.7 mm. in diameter, was made to flow under a pressure of 50 cm. of water. It was so adjusted as to impinge on the skin of the fish at right angles to

its surface and was not believed to be strong enough of itself to move the fish as a whole. It stimulated all those animals in which the skin was still intact and failed invariably where this was not the case. Apparently the current, like the drops of water, is limited in its activity to the integumentary terminals.

The responses called forth by the vibration of the aquarium as a whole are by no means so simple as those that have just been described. When the fish is near the surface, it is quite clear that the skin is vigorously stimulated, for in the four groups where their integument is intact, namely, 1 to 4 inclusive, responses occurred in large numbers. But it is also clear that these vibrations affect the ear, for the percentage of response is very high in group 6, in which the ear is the only receptor present, as well as in all other groups in which the ear is intact. There were no responses in groups 7 and 8, the only ones the representatives of which lacked effective skin and ears. We therefore conclude that the swaying back and forth of the whole mass of water in the aquarium stimulates vigorously both the skin and the ears of fishes near the surface of the water. The stimulus to the skin in this case is probably the same as that produced by a local current of water or by the falling of drops of water, but the stimulus to the ear is in our opinion not like that produced by the whistle. The whistle we believe to give rise to a true auditory stimulus but the slow rocking of the aquarium probably does nothing of the kind. It rocks the fish lightly in the water and it very likely thereby stimulates that portion of its ear that has to do with equilibrium; hence the response of the animal. If this supposition be true, the whistle, we believe, stimulates the saccular portion of the ear and the rocking of the aquarium the utricular portion of this organ. When the fish is well below the surface of the water in the aquarium, the slow swinging of the whole mass of water affects the animal much as when it is at the top, except that the effect is less in degree. This is clearly seen by comparing the two columns of figures in table 1 under "vibrations of whole aquarium."

Finally the responses of the fishes to the impact of the leaden ball on the slate end of the aquarium also involve both skin and ears. This is clearly seen from the records for groups 4 and 6 in the appropriate column of table 1. Moreover in all other groups in which the skin or the ears are effective, responses were observed, whereas in those in which these two organs were eliminated, groups 7 and 8, no responses appeared. Further, where skin and ear act together, groups 1 and 2, the percentages of the responses are unusually high as compared with

those in which only one of these two sense organs is present, groups 3 to 6 inclusive. These statements give ample evidence that the blow of the ball causes a stimulation of both the skin and the ears of Amiurus, but we are unable to state what part of the ear is stimulated. It seems to us probable that it is the same part that is acted on by the whistle in contrast to the part stimulated by the rocking of the aquarium as a whole, but on this point we have no conclusive evidence.

As aconsequence of the preceding discussion it might be concluded that the various mechanical stimuli thus far considered affected Amiurus through only two of the three sense organs under consideration, the skin and the ears, and that the lateral-line organs are not influenced by these stimuli at all. But a renewed inspection of the table will show that this is probably not the case. It is true that when the skin and ears are eliminated and only the lateral-line organs are left, group 7, the fishes failed completely to respond to any of the mechanical stimuli tried. But it is our belief that this does not show that the lateral-line organs are uninfluenced by these stimuli. We have been led to believe that the impulses from the lateral-line organs of Amiurus are inhibitory and not excitatory in nature. Evidence for this view can best be seen in the column of records for blows from the leaden ball. Thus with this form of stimulation when only the skin is intact, group 4, the percentage of response is 27, but when both skin and lateral-line organs are effective, group 3, the percentage falls to 11. When only the ear is intact, group 6, the percentage of responses is 73 but when the ear is combined with the lateral-line organs, group 5, the percentage drops to 33. Hence the presence of lateral-line organs in both cases reduces considerably the percentage of responses.

Much the same kind of evidence, at least so far as the ear is concerned, is to be seen in the responses to the vibrations of the aquarium as a whole. Thus in surface water the fishes responded to this stimulus through the ear alone, group 6, to the extent of 94 per cent, but through lateral-line organs and ear together, group 5, to only 80 per cent. In mid-water the response through the ear, group 6, was 26 per cent, through the lateral-line organs and ear, group 5, only 22 per cent. Here too the presence of effective lateral-line organs reduced in each instance the percentage of response.

But the same does not seem to be true of the skin and lateral-line organs. In this combination with the fish near the surface of the water the two organs, group 3, appear to be slightly more effective than the skin alone, group 4, and with the fish in mid-water they are de-

cidedly so. The records from which these particular numbers are drawn, however, are much more diverse than those for other parts of the table and we believe that the unconformity just pointed out is due rather to our inability to control quantitatively this form of stimulus than to any real grounds for difference.

It might also be urged that on the assumption that lateral-line impulses inhibit, there should be less response when all three organs are present, series 1, than when only the skin and ears are present, series 2. But the differences as shown in the table are so slight and so variable as to make them really negligible. In general fishes that have been operated upon, series 2, should be expected to be less delicately responsive than those that were normal, series 1. This together with the fact already stated, that it is impossible to control in a quantitative way the vibrations of the aquarium as a whole, is probably the explanation of the slight discrepancies under discussion. Hence we believe that these records in reality afford no serious obstacle to the opinion already expressed and supported from other sides that the lateral-line impulses inhibit to some degree the responses called forth through the skin and the ears. Granting this, we conclude that, notwithstanding the fact that no direct responses are obtained through the lateral-line organs, these receptors are nevertheless stimulated by the blows of the leaden ball and the vibration of the aquarium as a whole.

## RESPONSES TO SOUND

Although the responses of Amiurus to the whistle, as shown in table 1, seem to us to afford satisfactory evidence of the capacity of this fish to hear, for it responds to these sounds only when the ear is intact and never when it is eliminated, we nevertheless attempted to try out this question in other ways. The most satisfactory of these involved the use of a special piece of apparatus for which we are indebted to Prof. W. C. Sabine. This consisted of a series of seven alternating current generators with their armatures on a common shaft driven by an electric motor of ten horse-power. It yielded alternating electric currents of 43, 86, 172, 344, 688, 1376 and 2752 cycles per second. Any of these currents could be led separately into a telephone whence would emerge a tone having a pitch which in complete vibrations per second corresponded to the number of cycles. The tones yielded by the telephone were musical tones in which harmonics were unmistakably present.

To adapt this apparatus to our work with the fishes, we set it up in a basement room about 120 feet distant from that in which the aquarium was located. In this way the vibrations caused by the running of the apparatus which was of heavy construction and weighed not far from half a ton, were prevented from reaching the aquarium. By means of a cable the currents from the generator were conducted to the aquarium, where the telephone was situated. This had been slipped into a tight rubber cover, such as is used for a child's gas balloon, and with this protection it could be lowered into the water of the aquarium. By means of a switch (fig. 2) the activating current could be turned on, thus producing in the telephone a tone of a given pitch, which could be heard faintly in the air or better by applying the ear to the wall of the aquarium.

Fishes in pairs and representing the eight classes already described were subjected to these tones at intervals of not less than two and a half minutes and their responses noted. The details of the method of procedure were as follows: A pair of blindfolded fishes, appropriately prepared so far as receptors were concerned, were put in the small cage of the general aquarium, the telephone hanging in the water at one end of this cage. After the fishes had become accustomed to their location, they were watched and when one swam past the telephone so as to be within at least 10 cm. of it, the telephone was sounded and a record made of the response. After this, two and a half minutes were allowed to elapse as a resting period for the fish before it was subjected again to another trial. Work was continued in this way till five trials had been made on each of the two fishes. The results of these ten trials with the eight classes of fishes and the seven pitches are given in table 2, in which each number represents the number of responses in a total of ten trials for each combination.

A glance at table 2 shows at once the freedom with which the fishes responded to the tones from the telephone. We believe that this response is due to the tone itself and not to other possible disturbance. We have shown elsewhere (Parker and van Heusen, '17), however, that Amiurus is open to stimulation by the current from an electromagnet and as such a mechanism is concerned with the production of the tone in the telephone it is important to ascertain what influence it may have on the fish. To test this matter we removed the vibrating disc from the telephone and having inserted the latter in its rubber bag, we submerged it and tried it out with the fishes in the range of vibrations from 43 to 2752. To none of these did the fishes respond, though they were

immediately reactive to the tone from the telephone on reinserting the disc. We therefore concluded that the responses of the fishes to the sounding telephone under water are to the tones proper and not to any accompanying electrical disturbance.

A study of table 2 brings out two aspects of the question at hand: the relation of the responses to pitch and the various receptors concerned. In the matter of pitch it is quite clear that the fishes are much more generally stimulated by tones of low pitch than by those higher in the scale. Thus under every condition where response occurred at all the fishes reacted more generally to a pitch of 43 than to one of 86, and to 86 more than to 172 and so forth, except in series 3 where the reactions are uniform and in series 5 where a slight but insignificant irregularity occurs. Moreover, while every series in which there is any response at all includes responses to the lowest pitch, 43, no series exhibits responses to the highest two pitches, 1376 and 2752. The nature of the response is also different for the different pitches. In making records of the responses we recognized three types among them: a jump or spring of the fish forward, a sharp turn and a vibratory movement of the fins. These three types correspond, we believe, to degrees of stimulation, a jump or spring indicating the most vigorous stimulation and a fin movement the slightest stimulation. The proportion of these different types of responses in the reactions of normal fishes, table 2, series 1, are given in table 3.

TABLE 2

*Responses of eight classes of fishes to tones at octave intervals from 43 to 2752 complete vibrations per second. Each number represents the number of responses in ten trials, five on each of two fishes*

| | PITCH OF TONES IN COMPLETE VIBRATIONS PER SECOND | | | | | | |
|---|---|---|---|---|---|---|---|
| | 43 | 86 | 172 | 344 | 688 | 1376 | 2752 |
| 1. Skin, lateral-line organs and ear | 10 | 9 | 7 | 6 | 4 | 0 | 0 |
| 2. Skin and ear | 8 | 7 | 4 | 1 | 0 | 0 | 0 |
| 3. Skin and lateral-line organs | 2 | 2 | 2 | 0 | 0 | 0 | 0 |
| 4. Skin | 6 | 4 | 3 | 0 | 0 | 0 | 0 |
| 5. Lateral-line organs and ear | 5 | 3 | 5 | 1 | 0 | 0 | 0 |
| 6. Ear | 10 | 8 | 7 | 4 | 1 | 0 | 0 |
| 7. Lateral-line organs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8. None | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Here it will be seen that the spring or jump is characteristic of the

lowest pitch, that the turn is excited chiefly by the next higher pitch and that the fin movements are called forth in the main by still higher pitches. These several lines of evidence place beyond doubt the conclusion that Amiurus responds more freely to tones of a lower than of a higher pitch.

As to the receptors concerned in these reactions, table 2 offers important evidence. As is shown in series 6, the ear alone calls forth almost as much response as it does in conjunction with the skin and the lateral-line organs, series 1. The skin alone, series 4, is also receptive though to a less degree than the ear. As might be expected, the skin and ear together, series 2, show a high receptivity. The lateral-line organs,

TABLE 3

*Numbers of the three types of responses, spring, turn and fin movement, that make up the total responses of normal fishes to tones at octave intervals from 43 to 2752 complete vibrations per second. See table 2, series 1*

| | PITCH OF TONE IN COMPLETE VIBRATIONS PER SECOND | | | | | | |
|---|---|---|---|---|---|---|---|
| | 43 | 86 | 172 | 344 | 688 | 1376 | 2752 |
| Springs | 7 | 2 | 1 | 1 | 0 | 0 | 0 |
| Turns | 2 | 6 | 2 | 2 | 3 | 0 | 0 |
| Fin movements | 1 | 1 | 4 | 3 | 1 | 0 | 0 |
| Total responses | 10 | 9 | 7 | 6 | 4 | 0 | 0 |

series 7, give no direct evidence of acting as receptors, but a comparison among the other series shows that there is reason here as in the tests described on the preceding pages to regard the lateral-line organs as subject to stimulation but as originators of inhibitory, not excitatory impulses. Thus the numbers of responses exhibited by fishes in which the skin is the only receptor present, series 4, fall off when the lateral-line organs are also present, series 3. The same is true of the ear, series 6, and the lateral-line organs and ear, series 5. The fact that series 2, in which the skin and ear are effective, falls behind series 1, in which in addition to these two organs the lateral-line organs are present, is due, we believe, to the same cause as that which we pointed out in discussing table 1, namely that series 2 is composed of fishes that have been operated on and are therefore less responsive than normal individuals, series 1. Granting this assumption it may be concluded that tones stimulate all three sets of organs, the ears and skin producing excitatory impulses and the lateral-line organs inhibitory ones.

The range of receptivity is different for the different organs. With the ear, series 6, it extends from 43, where the responses are most abundant, to 688, where they are least numerous. The skin, series 4, has a more restricted range than the ear, giving rise to responses from 43 to 172 in a diminishing series. It is difficult to state over how wide a range in pitch the lateral-line organs are stimulated, but it is probably between that of the skin and the ear. Thus a difference in range of pitch is certainly true for the ear and skin and probably so for the lateral-line organs. The ear, though restricted to a lower range than the whole series of tones that we employed, is not so much restricted as the skin and probably the lateral-line organs. We therefore conclude that Amiurus responds to tones somewhat by means of the skin and lateral-line organs but principally by means of the ears.

This conclusion is supported by the results of local stimulation. As a fish swims slowly past the telephone, it is possible to produce the sound at such a moment as to have it impinge chiefly upon either the head of the animal including the ear or the posterior region of the trunk. When these two methods of application were employed at pitch 43, nine responses out of ten were obtained near the head and two out of ten near the tail. At pitch 86 seven out of ten were obtained near the head and none near the tail. Thus these rather crude attempts at local stimulation support the conclusion already stated that the ear is the chief organ concerned with these responses.

## DISCUSSION AND RESULTS

As our work has proceeded we have been more and more impressed with the great sensitiveness of Amiurus to mechanical stimuli, and our impressions on this point agree entirely with those of Sumner ('07) and of Maier ('09) quoted in the earlier part of this paper. If the water in the aquarium in which our experiments were conducted was not changed for some time, the fishes commonly frequented the surface, snapping air. Blindfolded individuals in such position would dive at once into deeper water at the movement of a chair on the concrete floor of the aquarium room, the slamming of a door in a distant part of the building, the sound of the human voice or the clapping of hands. These stimuli were generally not effective in mid-water. Nevertheless when blindfolded fishes were floating or quietly swimming in the deeper water, the lightest tapping of the finger on the slate end of the aquarium would cause them to swim downward. All these incidental observations and

many more of a like kind have convinced us that Amiurus is extremely responsive to mechanical stimuli.

That the skin in Amiurus is an important organ in the reception of these stimuli, as was maintained by Kreidl ('95) for the goldfish, is unquestionably true. As shown in table 1, series 4, the skin is stimulated by the dropping of water on the surface of that in the aquarium, by water currents, by the vibration of the whole body of water and by the disturbances produced through the impact of a leaden ball on the slate end of the aquarium. It is also stimulated by the lower tones from the telephone, table 2, series 4, from 43 to 172, but not by 344 or higher tones, including the whistle in the air, table 1. It therefore seems clear that the skin of Amiurus is open to mechanical stimulation of a wide range including sounds of low but not of high pitch. This great sensitiveness of the fish skin to mechanical stimulation is doubtless due to the fact that it is bathed with water and may therefore possess a delicacy far surpassing that of the skin of terrestrial animals, whose dry surfaces are more adapted to reflecting than receiving such timuli. Since Kreidl's original statement almost every investigator has added confirmatory evidence to his view that the integumentary sense organs of fishes are freely and normally open to mechanical stimulation including sound.

The function of the lateral-line organs in fishes is by no means so clearly understood as that of the skin and the opinions already expressed on this subject are strikingly diverse. The discovery of the nervous nature of these organs by Leydig ('50, '68) was followed by a great variety of speculations as to their probable function. Schulze ('70) supposed them to be stimulated by the movement of the whole mass of water about the fish and by waves longer than those that stimulate the ear, an opinion shared by Malbranc ('76). Merkel ('80) regarded them in the nature of tactile organs, while Emery ('80), Mayser ('81), Bodenstein ('72) and P. and F. Sarasin ('87–90) looked upon them as primitive ears.

Experimental evidence as to the function of these organs is rather scanty. Bugnion ('73) showed that in Proteus they were not stimulated by chemicals, but were receptive to mechanical influences, and de Sède ('84) claimed that fishes deprived of their lateral-line organs avoided obstacles in the water less successfully than normal fishes did. Both Bateson ('90) and Nagel ('94) confirmed the view of Bugnion that these organs are not stimulated by chemical means. Fuchs ('94) gave some indirect evidence to show that they were bought into activity

by change of pressure. Richard ('96) believed that they were the means of controlling the production of gas in the air bladder and Bonnier ('96) advanced the view that they helped to orient a fish in reference to a center in the water from which vibratory disturbances emanated. Lee ('98) thought them concerned with equilibrium. Parker ('05) believed them to be stimulated by vibrations of low frequency and Hofer ('08) maintained that they were receptors for local currents.

The evidence presented in this paper points quite conclusively to a vibratory stimulus. The dropping of water in the aquarium and slight currents of water have no effect on these receptors in Amiurus, but are pronounced stimuli for the skin. As already pointed out, a slow rocking of the aquarium as a whole, the disturbance caused by a blow from a leaden ball delivered on the slate end of the aquarium, as well as the lower range of tones from the telephone are stimuli for the lateral-line organs of Amiurus though the impulses thus produced are of an inhibitory rather than an excitory nature. Sound vibrations from the telephone above 688 seem to have no effect on these organs nor does the shrill noise of a whistle sounded in the air. We therefore conclude that in Amiurus the lateral-line organs are stimulated by water vibrations from a few per second to several hundred in that interval. In this sense these organs do resemble primitive ears.

This conclusion is in substantial agreement with that arrived at by Parker ('05) in his study of the lateral-line organs of Fundulus and other marine fishes, in which the stimulus was shown to be a low vibration of approximately six per second. It has recently been denied by Hofer ('08), but on what we regard as insufficient grounds. Hofer worked chiefly on Esox, but with a plan of experimentation that seems to us much too limited. In preparing his fish for tests he did nothing more than exclude part or all of the lateral-line system without reference to the closely related organs, the skin and ears. In our opinion he should also have tested fishes in which these two organs were rendered ineffective and the lateral-line organs left intact. We have already pointed out the intimate and over-lapping character of the fields of the skin, lateral-line organs and the ears, and a test that is based entirely on the absence of one in the presence of the other two and that never takes into account what that one alone might do seems to us insufficient. We do not deny that in Esox the lateral-line organs may respond to currents, though we regard the evidence very meagre; but if these organs do so respond, there is no proof in this that the corresponding organs in Fundulus and other marine fishes as worked out by Parker and in

Amiurus as shown in this paper, do not respond to water vibrations of a low rate. To deny this on the reactions of Esox seems to us unwarranted. Hofer ('08, pp. 139, 146) himself states that though he worked on Cyprinus, Trutta, Cottus, Squalinus and Esox the last was the only one that gave good results of the kind he described. In view of what has been found for Fundulus and other fishes as well as for Amiurus, it seems to us that, if the lateral-line organs of Esox are found to be receptors for water currents, they may be in this respect distinctly exceptional, for in the majority of fishes thus far tested this has not been found to be the case. In our opinion Schulze was right in his belief that vibrations of a low rate are the normal stimuli for the lateral-line organs. Whether he was also correct in assuming that mass movements in the surrounding water, such as are still seen in local currents and so forth, are also stimuli for these organs remains, we believe, to be ascertained.

The question of hearing in fishes has had a longer history than that of the function of the lateral-line organs and is correspondingly more complicated. The presence of internal ears in fishes as demonstrated by Casserius in the seventeenth century and the subsequent study of these organs by Geoffroy, Scarpa, Comparetti, Hunter and others led to the general conclusion that fishes possessed a sense of hearing, a conclusion accepted by most zoölogists until very recent times. Cyon ('78) seems to have been the first to question this belief; he based his skepticism on experiments with lampreys. These fishes, according to him, were not responsive to sounds and the loss of the ear was followed with disturbances in equilibrium. Cyon ('78, p. 95) therefore concluded that the so-called auditory organs of the cyclostomes were really organs of equilibrium.

Some years later Kreidl ('95) undertook experiments on the function of the ears in goldfishes. He concluded that normal goldfishes never responded to sounds produced either in the air or in the water though they would react to a blow given to the cover of an aquarium. After having been rendered especially sensitive by strychnine, they would respond to slight noises, but the response was as vigorous after the removal of the ear as before. He therefore concluded in this and in a later work ('96) that sound-waves stimulated the skin and not the ears of fishes. This conclusion was supported by Lee ('98), who experimented on a number of fishes without getting any evidence to show that they possessed a sense of hearing. Körner ('05; '08) studied some twenty-five species of fishes that had been for a long time in aquaria

and was unable to get any response to a "cri-cri" (a child's toy for producing a noise) sounded under water. He therefore reaffirmed Kreidl's conclusion. Negative results were also obtained by Marage ('06), who experimented on some six species of fish, and by Bernoulli ('10) who worked upon three kinds. Finally Maier ('09) tested nine species of salt-water fishes and eleven species of fresh-water forms but with negative results except in the case of Amiurus, which, as already noted, he recorded as responsive to a sharp whistle.

As opposed to this considerable body of negative evidence may be placed the observations of Zenneck ('03) on three species of fresh-water fishes, which were found to respond to an electrically driven bell submerged in the water. Lafite-Dupont ('07) also found that of the six species studied by him four were responsive to taps on the wall of the containing vessel. Neither Zenneck nor Lafite-Dupont determined whether the responses that they observed were dependent upon the skin or the ear, and hence it is entirely possible to interpret their results in support of Kreidl's original contention that all such reactions are due to integumentary receptors and have nothing whatever to do with hearing.

As opposed to the view that fishes respond to sounds exclusively through the skin may be cited the work of Parker ('03 a; '03 b) on Fundulus, in which it was shown that though the skin is sensitive to vibratory movements of the water, responses to this form of stimulus fall off so considerably after the eighth nerves have been cut that it is difficult, as Hensen ('04) pointed out, to understand why hearing should not be attributed to such animals. A similar conclusion was reached by Bigelow ('04) in his study of the goldfish, and like results were obtained by Parker on the dogfish ('09, '11 a) and the squeteague ('10b). Previous to this later work Piper ('06 a, '06 b) had shown that an action current was to be observed on the eighth nerve of the pike and the eel when their ears were subjected to sound vibrations. Yet notwithstanding this considerable body of positive evidence, Watson ('14, p. 393), in one of the few summaries of this subject, finds it difficult to arrive at any satisfactory conclusion on the question, *do fish hear?*

Many of the contradictory statements that occur in the literature of this subject and that have confused students are due, we believe, not so much to mistaken observation as to unsuspected differences in the way in which the fishes are managed in the various tests. Thus, in Amiurus we soon learned that the way to obtain the most delicate responses was to leave the fishes for several days in the aquarium ar-

ranged for the trials till they were thoroughly at home, and then to test them when they were swimming or floating near the surface of the water. Such conditions insured the greatest certainty of response. If an aquarium containing blindfolded fishes is watched from a distance, the fish are usually seen to be swimming near the top. On walking toward it with a customary step all the fish usually swim to the bottom. There they seem to be as much composed as near the top, but it would be almost impossible under these circumstances to get responses from them to many of the more delicate mechanical stimuli that we have used. The experimenter must wait quietly some time till the fish have again regained the upper water before tests of a trustworthy kind can be made. And during the making of these tests the utmost precaution must be exercised not to subject the fish to any form of stimulation except that which for the moment is being tested.

In our opinion many of the negative results recorded in the literature on fish hearing are due not to the absence of the sense of hearing on the part of the fish but to attempts to get responses from fishes that were in no condition to give them. This kind of inexpertness, we feel sure, runs through many of the records of the past. It is almost inconceivable to us how Körner ('05) could have tested out in the way he described twenty-five species of fishes and got negative results that could be relied upon. Edinger ('08) is entirely correct in his insistence on the importance of a thorough acquaintance with a given fish and the avoidance of the unnatural in experimenting with it.

Another source of confusion in the results of various workers on this problem is in the relation of the sounds to the aquarium. To make a test with certainty it is absolutely necessary to produce sounds that really reach the fishes. Many sounds, like the clapping of hands, the blowing of whistles and so forth, made in the air in the neighborhood of an aquarium may never penetrate the water at all or do so to so small an amount as to have no effect on the fishes. As is well known, most sounds produced in the air fail to pass into the water but are reflected in large part from its surface. Conversely most sounds made under water fail to escape to any considerable degree into the air but are limited to the water. Hence it is that in many tests negative results may have been obtained because in reality the sound failed to reach the fish (Parker, '11 b). We doubt very much if the noise from the hand clapping and striking together of stones as carried out by Lee ('98) ever reached the fishes in the aquarium. And we have equal doubts of the efficiency of sounds made in air by Marage ('06) or of the pistol

shots recorded by Bernoulli ('10, p. 643). Devices for producing sound in an aquarium must be applied directly to the wall of that vessel or better submerged in the water itself, as has been practised by Zenneck ('03), Körner ('05), Maier ('09) and others.

We have shown in Amiurus that the fish responds much more generally to tones of low than of high pitch and it is our belief that in general fishes are more responsive to the long than to the short waves. In not a few instances it is quite possible that the pitch used by the given investigator was too high for the fishes and that the negative results obtained are to be explained not as a consequence of the lack of hearing on the part of the fishes, but because the stimulus was beyond their range. This may have been the case with Körner ('05), who employed a "cri-cri," which in the forms familiar to us always gives out a high-pitched note.

It seems to us that the negative evidence that has been gathering around the question of fish hearing is to be accounted for chiefly through defects in technique; either the fishes were not in a condition to respond, or the stimulus was prevented from actually reaching them, or if it did reach them, it was beyond their range. Such explanations, however, do not make clear why Zacharias ('06, p. 373) in a general article on hearing in fishes, should have quoted Zenneck ('03) and Bigelow ('04) as having obtained only negative results.

Some investigators in this field of research, such as Bateson ('90) and Kreidl ('95), have suggested that the responses of the fish to so-called sounds were as a matter of fact not so much to the sound proper as to the shock that may accompany the sound particularly when it is a loud one. We are disinclined, however, to accept this distinction, for to us an explosive sound and its shock are one and the same movement. Such sounds are very different from tones but we nevertheless speak of them as being heard by ourselves. Hammer blows and gun reports are just as much heard by us as the tone from a tuning fork. We therefore do not admit that explosive noises with their shock quality are not legitimate means of testing the sense of hearing in fishes. What we are concerned in ascertaining is whether these disturbances stimulate the ears of fishes or only their skin. In the latter case it is not hearing, in the former it is. Amiurus in responding through the ear alone in 73 per cent of the trials to blows of a leaden ball on the wall of the aquarium and in only 33 per cent of the trials after the eighth nerve had been cut gave in our opinion perfectly conclusive evidence of hearing, notwithstanding the fact that the blows had the quality of shock.

The difficulty of fully appreciating this situation has probably added confusion to the minds of not a few investigators. Thus the negative evidence in the question of fish hearing seems to us to be without great weight, for much of it has been brought together under circumstances where it was improbable that the fishes would have yielded any results whatsoever. On the other hand the positive evidence appears to us so strong and convincing that we fail to see how hearing in the usual acceptation of the term can be denied to many fishes; Fundulus (Parker, '03 a); Carassius (Bigelow, '04); Mustelus (Parker, '09, '11 a); Ammocoetes (Parker, '10 a); Cynoscion (Parker, '10 b) and Amiurus. Hence we venture to predict that hearing will be found a common function for the ears of fishes.

TABLE 4

*A comparison of the ranges of the skin, lateral-line organs and ears of Amiurus to various mechanical stimuli*

| | STIMULI | | | | | | |
|---|---|---|---|---|---|---|---|
| | Drops of water | Currents of water | Vibrations of whole mass of water | Blows from leaden ball | Low-pitched tones | High-pitched tones | Whistle in air |
| Skin | x | x | x | x | x | 0 | 0 |
| Lateral-line organs | 0 | 0 | x | x | x | 0 | 0 |
| Ear | 0 | 0 | x | x | x | x | x |

The three sets of sense organs with which this paper has been concerned have been regarded by many workers as genetically related, and that relation is quite as clearly indicated by the nature of their appropriate stimuli as by other means. This is summarized in table 4, where it will be seen that the skin, which is the most primitive of the three receptors, is stimulated only by the grosser forms of mechanical disturbances. This is followed by the next derivative, the lateral-line system, whose range is limited to the less gross stimuli that affect the skin. And this in turn is followed by the ear, the most highly evolved of these three sets of organs, whose range of receptivity runs higher than that of the lateral-line organs and not so low as that of the skin. Thus the line of descent of these three sets of receptors suggested by their ontogeny is also in accordance with the sequence laid down by their receptive capacities. It is interesting to note in table 4 that each receptor has not a field of its own but that the three fields overlap in

a way that one would expect on the supposition that the skin had given rise to the lateral-line organs and these to the ear.

## SUMMARY

1. The skin of Amiurus is stimulated by the dropping of water, by water currents, by a slow vibratory movement of the whole body of water, by the impact of a leaden ball on the slate wall of an aquarium, by the lower tones (43, 86 and 172 complete vibrations per second) from a submerged telephone, but not by the higher tones (344, 688, 1376 and 2752 complete vibrations per second), nor by a whistle blown in the air. The responses to these stimuli are locomotor.

2. The lateral-line organs of Amiurus are stimulated by a slow vibratory movement of the whole body of water, by the impact of a leaden ball on the slate wall of an aquarium, by the lower tones from a submerged telephone (43, 86, 172 and 344 vibrations), but not by the dropping of water, by water currents, by the higher tones from the telephone (688, 1376 and 2752 vibrations), nor by a whistle blown in the air. The responses to these stimuli are inhibitions of those initiated through the skin and the ear.

3. The ear of Amiurus is stimulated by a slow vibratory movement of the whole body of water (utriculus?), by the impact of a leaden ball on the slate wall of an aquarium (sacculus?), by certain tones from a submerged telephone (by 43, 86, 172, 344 and 688, but not by 1376 and 2752 vibration), by a whistle blown in the air, but not by the dropping of water nor by water currents. The responses to these stimuli are locomotor.

POSTSCRIPT. Since the preparation of this paper for press there has come to our notice an article on the hearing of fishes, particularly Amiurus nebulosus, by O. Körner (Zeitschr. Ohrenheilkunde, lxxiii, 257, 1916). In this paper Körner states that no evidence could be found that the ear of Amiurus was stimulated by sound. He confesses his inability to explain the responses observed in this fish by Maier. His negative results in this instance, as in his earlier work, seem to us to be due to faulty technique.

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# NOTE ON THE REVERSAL OF RECIPROCAL INHIBITION IN THE EARTHWORM

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Sherrington first showed that in vertebrates under the action of strychnine, practically all skeletal muscles, inclusive of antagonistic muscles, may be reflexly thrown into contraction simultaneously (1). This necessarily involves the abolition of reciprocal inhibition by converting inhibitions into excitations.

It has been shown that a single excitation in the earthworm elicits a shortening of the worm anterior to the point of stimulation and a lengthening posterior to that point (2). It was proven by Garrey and Moore that this involves reciprocal innervation since a contraction of the circular muscles is associated with relaxation of the longitudinal ones. It is generally recognized that the earthworm is the simplest form in which the synapse has been described. It seemed possible, therefore, that the neurone relations in this form might be affected by strychnine in the same fashion as in vertebrates and in this way the inhibition of the posterior longitudinal muscles be converted into excitation.

In order to test this point, we made use of the earthworm preparation described by Garrey and Moore (2). In our experiments the earthworms were put into strychnine sulphate solution, strength 1 : 10,000, and allowed to remain until the spasms showed that the strychnine had taken effect. A suitable length of the worm was then removed and tested by means of electrical and mechanical stimulation of the integument. The results, illustrated by figure 1, showed complete reversal of the normal posterior response to stimulation, viz.: shortening instead of lengthening of this portion as a result of contraction replacing relaxation in the longitudinal musculature.

Normally the impulse passing backwards along the nerve cord causes a relaxation of the longitudinal muscles, in other words, a true reciprocal inhibition. In the strychninized worm stimulation causes con-

traction of all the muscles both circular and longitudinal. Since the longitudinal muscles are the more powerful the net result is a shortening of the worm in a rigid condition. The result obtained means, then, that the impulse passing backward along the cord, which normally causes a relaxation of the longitudinal muscles, is converted by strychnine into an excitation which causes their contraction and hence the reciprocal inhibition is reversed by strychnine.

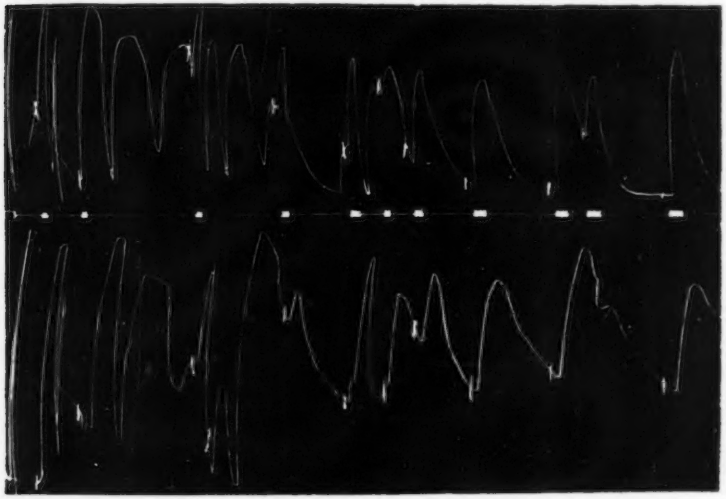

Fig. 1. Effects of faradic stimulation of the integument in the middle of a strychninized worm preparation. The upper tracing is a record of the contractions of the anterior half, and the lower tracing of the posterior half of the preparation. The stimulation periods are shown on the middle line.

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# ATTEMPTS TO PRODUCE EXPERIMENTAL THYROID HYPERPLASIA

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The following series of experiments were carried out at the suggestion of Doctor Carlson to determine certain factors that have been reported to induce thyroid hyperplasia in man and experimental animals.

## I. HIGH PROTEIN DIET

Attention was called to the effect of high protein diet on the thyroid by C. Watson in 1904. At this time he found that prolonged administration of raw meat to healthy fowls induced certain changes in these glands making them different histologically from the glands of fowls on an ordinary diet. He confirmed these results in 1906 by feeding rats. Later ('07) he found that wild rats kept in captivity and fed on a bread and milk diet developed hyperplastic thyroids.

Reid Hunt ('11) reported finding large vascular thyroid glands in mice fed on a diet of oatmeal and liver. The same year Marine and Lenhart in their observations on brook trout found goitre endemic in all hatcheries where the salmonidae were artificially fed. Its severity was related to hygienic conditions, food, water supply and degree of crowding.

An altered condition of the thyroid with an increased cellular element was induced by C. Watson ('12) in rats and mice by feeding a diet of flour alone.

Marine ('14) produced hyperplasia of the thyroid in brook trout by feeding liver and heart. He was able to bring about an involution of this condition by changing the diet to fresh sea fish.

Bensley ('14) noticed that the opossum developed hyperplasia in a relatively short time after being put into captivity. He was able to control the degree of hyperplasia in this animal at will by diet.

Fresh and stale liver was fed young rats by Marine ('15). All his rats gained in weight. Those receiving liver one, two and three days

old showed distinct hypertrophy as judged by the reduction in amount of stainable colloid and the increase in the size of the thyroid cells. Those given fresh liver and liver four, five and six days old had normal or nearly normal glands.

The rats in the following experiments were carefully weighed at the beginning of the experimental feeding period and again before killing at the close of the period. All rats were anesthetized and killed by bleeding. The thyroids were removed as quickly as possible and weighed in moist weighing bottles. They were then fixed in formalin Zenker or acetic osmic bichromate. The cages were cleaned and fresh water given daily. (Table 3 forms an exception of which mention will be made later.)

Bread soaked in milk was used as the standard diet and fresh liver and lean beef (muscle) was given for the high protein diet. The liver or muscle was usually mixed with a little oatmeal or bread crumbs since this enables the rat to live indefinitely. The full amount that the animal would eat each day was given but no more. The rats fed on a standard diet gained rapidly in weight as a rule while those fed on meat grew if young but if mature usually lost weight.

Histologically these glands presented a somewhat uniform appearance. Ordinarily there was a fair amount of colloid, the follicles were round and the cells slightly columnar. The glands of most animals on high protein diet showed a greater or less degree of hyperplasia. Mitosis was rarely seen. A few showed secretion antecedent with Dr. Bensley's new brasilin stain. Since the weight of the thyroid gland has been shown by the above author to be a criterion as to its degree of hyperplasia, I have made use of this method in drawing my conclusions.

I have placed alongside the actual weight of the gland in each case for purposes of comparison the weight as computed by the method given by Hatai based on the body weight of the rat. In each case the body weight at the time the animal was killed was used for computing the thyroid weight.

Table 1 represents the results from six full grown rats. Numbers 1, 2 and 3 received daily fresh liver ground up with a little oatmeal. Numbers 4, 5 and 6 were given bread soaked in milk. Number 6 for some unknown reason lost considerably in body weight but the thyroid weight was below normal showing that loss in body weight does not necessarily indicate a hyperplastic tendency of the thyroid gland.

Number 5 of table 2 showed a very hyperplastic gland in spite of the fact that it was rapidly gaining weight.

The animals represented in table 3 were kept in uncleaned cages. The excreta was allowed to collect in the cage and the drinking water of the animal became highly contaminated with fecal refuse. All the animals with one exception gained in weight during the experimental

TABLE 1

*The effect of a high protein diet on the thyroid in adult rats*

| NUMBER OF RAT | DIET | INITIAL WEIGHT | CHANGE IN WEIGHT | WEIGHT OF THYROID | | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|---|
| | | | | Actual | Hatai computed | |
| | | *grams* | *grams* | | | *days* |
| 1 | High protein diet | 252 | −32 | 0.0540 | 0.0344 | 34 |
| 2 | | 187 | − 9 | 0.0310 | 0.0294 | 26 |
| 3 | | 258 | −20 | 0.0300 | 0.0368 | 26 |
| 4 | Standard diet | 148 | − 3 | 0.0170 | 0.0252 | 25 |
| 5 | | 157 | 21 | 0.0210 | 0.0294 | 25 |
| 6 | | 273 | −73 | 0.0240 | 0.0322 | 35 |

TABLE 2

*This group received a similar diet to the rats represented in table 1*

| NUMBER OF RAT | DIET | INITIAL WEIGHT | CHANGE IN WEIGHT | WEIGHT OF THYROID | | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|---|
| | | | | Actual | Hatai computed | |
| | | *grams* | *grams* | | | *days* |
| 1 | High protein diet | 112 | 5 | 0.0110 | 0.0214 | 32 |
| 3 | | 103 | 37 | 0.0190 | 0.0243 | 32 |
| 5 | | 120 | 48 | 0.0750 | 0.0284 | 32 |
| 7 | | 123 | 23 | 0.0300 | 0.0253 | 32 |
| 9 | | 320 | −33 | 0.0350 | 0.0421 | 32 |
| 11 | | 317 | −52 | 0.0300 | 0.0401 | 32 |
| 2 | Standard diet | 113 | 25 | 0.0270 | 0.0243 | 32 |
| 4 | | 150 | 28 | 0.0300 | 0.0295 | 32 |
| 8 | | 158 | 26 | 0.0250 | 0.0294 | 32 |
| 10 | | 355 | −69 | 0.0250 | 0.0421 | 32 |

feeding. The average body weight of the rats on the high protein diet was about the same as that of the rats fed on a normal diet, yet the average thyroid weight was 0.0108 gram greater in the case of the former. The normal fed animals had an average thyroid weight considerably above the Hatai computed weight. This would indicate, as

McCarrison ('14) has reported, that there is some hyperplasia induced in rats fed on a normal diet and kept under unhygienic conditions. All the liver fed animals showed distinctly heavier thyroids with the exception of number 1 and this was a young, growing rat.

In table 4 all the meat fed rats were young and every one grew well on the diet. None showed marked hyperplasia. This would suggest that the young growing rat can thrive on a high protein diet and its thyroid gland is not affected as in the case of the more mature animals.

TABLE 3

*The effect of high protein diet and standard diet on the thyroid with the additional factor of unhygienic cages*

| NUMBER OF RAT | DIET | INITIAL WEIGHT | CHANGE IN WEIGHT | WEIGHT OF THYROID | | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|---|
| | | | | Actual | Hatai computed | |
| | | *grams* | *grams* | | | *days* |
| 7 | | 97 | 78 | 0.0230 | 0.0290 | 31 |
| 8 | | 110 | 77 | 0.0440 | 0.0310 | 40 |
| 9 | High protein | 177 | 18 | 0.0610 | 0.0316 | 37 |
| 10 | diet | 252 | −12 | 0.0550 | 0.0370 | 37 |
| 11 | | 137 | 82 | 0.0550 | 0.0344 | 40 |
| 12 | | 110 | 35 | 0.0280 | 0.0250 | 31 |
| 1 | | 119 | 57 | 0.0250 | 0.0290 | 31 |
| 2 | Standard diet | 137 | 63 | 0.0280 | 0.0325 | 40 |
| 3 | | 155 | 50 | 0.0270 | 0.0328 | 37 |
| 4 | | 240 | 15 | 0.0520 | 0.0353 | 37 |
| 5 | | 97 | 75 | 0.0350 | 0.0290 | 40 |
| 6 | | 155 | 31 | 0.0350 | 0.0304 | 31 |

Table 5 represents a fairly even litter of rats from Donaldson's laboratory, Wistar Institute. These animals were kept in an iodine-free room in Doctor Bensley's laboratory, thus eliminating any possibility of error which might be introduced by the presence of free iodine. For a high protein diet the rats were given lean beef (muscle). All the rats on this diet lost in weight and most of them showed distinct hyperplasia of the thyroid gland. The bread fed animals all gained in weight and none showed hyperplasia.

In table 6 I have made a summary of the results shown in tables 1 to 5. The average in each of the above groups with the difference between the average actual weight and the average computed weight of the thyroid is expressed. The average weight of the meat fed rats

in the five groups was 164 grams; of the bread fed, 179 grams. Yet the average weight of the thyroids of the meat fed rats was 0.0311; and that

TABLE 4

*The effect of a high protein diet on the thyroid in growing rats*

| NUMBER OF RAT | DIET | INITIAL WEIGHT | CHANGE IN WEIGHT | WEIGHT OF THYROID | | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|---|
| | | | | Actual | Hatai computed | |
| | | *grams* | *grams* | | | *days* |
| 7 | | 55 | 57 | 0.0150 | 0.0207 | 31 |
| 8 | | 80 | 25 | 0.0150 | 0.0197 | 31 |
| 9 | High protein | 90 | 35 | 0.0190 | 0.0225 | 31 |
| 10 | diet | 62 | 38 | 0.0200 | 0.0190 | 33 |
| 11 | | 68 | 54 | 0.0270 | 0.0222 | 33 |
| 12 | | 60 | 25 | 0.0130 | 0.0167 | 33 |
| 1 | | 114 | 68 | 0.0210 | 0.0300 | 31 |
| 2 | | 113 | 72 | 0.0240 | 0.0300 | 31 |
| 3 | Standard diet | 140 | 17 | 0.0280 | 0.0268 | 31 |
| 4 | | 147 | −7 | 0.0320 | 0.0243 | 17died |
| 5 | | 120 | 70 | 0.0350 | 0.0310 | 31 |
| 6 | | 170 | 7 | 0.0380 | 0.0290 | 31 |

TABLE 5

*The effect of a high protein diet on a litter of rats from Donaldson's laboratory kept in an iodine-free room*

| NUMBER OF RAT | DIET | INITIAL WEIGHT | CHANGE IN WEIGHT | WEIGHT OF THYROID | | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|---|
| | | | | Actual | Hatai computed | |
| | | *grams* | *grams* | | | *days* |
| 8 | | 145 | −10 | 0.0260 | 0.0235 | 56 |
| 11 | | 130 | −40 | 0.0270 | 0.0175 | 56 |
| 14 | High protein | 145 | − 5 | 0.0210 | 0.0243 | 56 |
| 15 | diet | 135 | −5 | 0.0140 | 0.0231 | 56 |
| 16 | | 150 | −60 | 0.0300 | 0.0173 | 49 |
| 2 | | 117 | 23 | 0.0190 | 0.0243 | 61 |
| 4 | Standard diet | 142 | 16 | 0.0190 | 0.0268 | 61 |
| 10 | | 132 | 23 | 0.0198 | 0.0268 | 61 |

of the bread fed was 0.0280. This shows an increase in weight of the thyroid gland of the animals fed on a high protein diet over that of the animals fed on a standard diet of bread and milk of 10 per cent.

These experiments show that a high protein diet, either fresh liver or muscle, in the course of a few weeks, produces in the adult white rat a distinct hyperplasia of the thyroid gland. The fact brought out by McCarrison ('14) is further emphasized, namely that unclean cages have a tendency to produce goitrous glands in rats.

TABLE 6

*Summary of the effects of a high protein diet on the thyroid, detailed in tables 1 to 5*

| GROUP | DIET | AVERAGE BODY WEIGHT | AVERAGE THYROID WEIGHT IN GRAMS | | DIFFERENCE IN THYROID WEIGHT |
|---|---|---|---|---|---|
| | | | Actual | Hatai | |
| | | *grams* | | | |
| I | | 212 | 0.0383 | 0.0335 | 0.0048 |
| II | | 187 | 0.0333 | 0.0303 | 0.0028 |
| III* | High protein | 194 | 0.0445 | 0.0313 | 0.0132 |
| IV | diet | 108 | 0.0182 | 0.0201 | −0.0019 |
| V | | 117 | 0.0236 | 0.0205 | 0.0031 |
| I | | 174 | 0.0234 | 0.0234 | |
| II | Standard diet | 197 | 0.0268 | 0.0313 | −0.0045 |
| III* | | 199 | 0.0337 | 0.0315 | 0.0022 |
| IV | | 172 | 0.0297 | 0.0285 | 0.0012 |
| V | | 151 | 0.0193 | 0.0259 | 0.0066 |

* The influence of unclean cages stands out markedly in those rats receiving standard diet as well as in those receiving a high protein diet.

## II. INFLUENCE OF FECES FROM GOITRE PATIENTS AND GOITRE DOGS

This series of experiments was undertaken in an attempt to confirm the findings of some recent investigators, namely, that excreta from human or animal subjects is an important factor in the production of goitre.

Marine and Lenhart ('10) found that in regions of endemic goitre the fish may also be affected. This was thought to be direct evidence that goitre was associated with water. Marine ('14) by experiments on brook trout came to the conclusion that goitre was non-infectious and non-contagious.

McCarrison ('11) gave goats water which came through sterilized soil mixed with feces of goitrous individuals. Many of the animals developed diarrhoea and 50 per cent of them showed enlargement of the thyroid. He came to the conclusion that hypertrophy of the thyroid was due to an infecting agent of goitre or some organic impurity

of water. Treatment of simple goitre in individuals by vaccines of coliform bacillus, a spore-bearing bacillus and a staphylococcus with success led him further to believe that the gland undergoes hypertrophy as the results of a stimulus which is commonly a toxic material absorbed from the alimentary tract, the enlargement of the thyroid being due to an effort on the part of the gland to resist some toxic agency. He also proposed intestinal antiseptics as a treatment for goitre. Bircher's work (quoted from McCarrison) on feeding feces with cooked rice and giving subcutaneous injections of feces to rats showed positive results. McCarrison thus concluded that goitre was caused by the toxin of a microörganism that finds its home in the intestine of man. Feeding non-goitrous feces, goitrous feces, aerobic organisms and anaerobic organisms from goitrous feces gave enlargement of the thyroid in rats as did keeping in unhygienic conditions (McCarrison, '14).

Messerli ('14) found that water from a goitrous region produced goitre in rats.

Rosenow ('14) reported the finding of a gram positive diplo-bacillus-like organism in the thyroid in twenty-five out of thirty cases of goitre in man and eight out of twelve cass of goitre in dogs.

Feeding pregnant goats feces, McCarrison ('16) obtained 100 per cent goitrous kids. He concluded that the toxic substance derived from the intestine of the mother produced goitre in the foetus.

Cats were used in the following experiments since these animals are seldom found to have goitre when brought into the laboratory or to develop spontaneous goitre when kept under laboratory conditions. Any goitrous condition occurring among them, therefore, while in the laboratory, might safely be considered to be due to experimental procedure.

All cats in tables 7 and 8 had the left thyroid removed a few weeks before the experimental feeding began. The gland was weighed and fixed in formalin Zenker. The cats in table 7 were given a solution of feces from a goitrous dog. About 100 cc. of this material was injected into the stomach with a stomach tube every second day and was retained. Animal number 5 was kept as a control.

Cats 9, 12, 14 and 17 in table 8 received a solution of feces from an exophthalmic goitre patient every second day. Cats 6 and 13 were given a solution of feces from a normal person. Cat 11 was used as a control.

These animals thrived on a normal diet in spite of the injection of fecal matter. Most of them gained in weight during the experimental

period. The difference in weight of the glands before and after feeding is not enough to be of any significance. The histological changes were negligible. The wide differences in environment and food that must exist among cats procured for laboratory work makes it impossible to describe a normal gland histologically. The two thyroids in the same animal are usually very similar as regards weight, amount and staining

TABLE 7

*Cats given feces from a goitrous dog*

| NUMBER OF CAT | BODY WEIGHT | THYROID WEIGHT | | CHANGE IN BODY WEIGHT | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|
| | | Left | Right | | |
| | *grams* | *grams* | *grams* | | *days* |
| 1 | 2060 | 0.165 | 0.165 | 1055 | 65 |
| 2 | 3780 | 0.225 | 0.215 | 660 | 65 |
| 3 | 3220 | 0.080 | 0.110 | 295 | 65 |
| 4 | 2560 | 0.275 | 0.150 | −820 | 35 |
| 5 | 2320 | 0.090 | 0.105 | 545 | 65 |

TABLE 8

*Cats given feces from normal individual and from an exophthalmic goitre patient*

| NUMBER OF CAT | BODY WEIGHT | THYROID WEIGHT | | CHANGE IN BODY WEIGHT | DURATION OF EXPERIMENTS |
|---|---|---|---|---|---|
| | | Left | Right | | |
| | *grams* | *grams* | *grams* | | *days* |
| 9 | 3000 | 0.105 | 0.108 | 150 | 75 |
| 12 | 2900 | 0.120 | 0.100 | −100 | 75 |
| 14 | 2720 | 0.155 | 0.190 | 480 | 90 |
| 17* | 3200 | 0.580 | 0.370 | 300 | 90 |
| 11 | 3040 | 0.210 | 0.200 | 260 | 90 |
| 6 | 4200 | 0.115 | 0.120 | −260 | 75 |
| 13 | 3280 | 0.125 | 0.145 | −340 | 90 |

* Had been suckling young.

properties of colloid, size of follicle and dimensions of the epithelial cell. Therefore, the individual comparison of the gland before feeding with the one after feeding was used as a criterion of histological change due to experimentation. No hypertrophy could be said to have been induced in these animals.

Focal infections such as may occur in tonsils, teeth, sinuses of the head and joints of the body, appear to bear some relation to goitre

(Billings, '14; Brown, '14; Buford, '15). Evans, Middleton and Smith ('16) have reported findings in a number of cases of goitre in endemic goitre regions in Wisconsin which lead them to believe tonsillar infection may be one means by which goitre may be induced. With this in mind an attempt was made to infect the tonsils of three cats by injecting virulent streptococci directly into the tonsil with a hypodermic syringe.

A bouillon suspension of virulent streptococci was injected into the tonsils of the cats while under an anesthetic. Several injections were made with an interval of seven days between each. In no case were we able to produce a tonsillar infection or thyroid hyperplasia by this means.

### III. UNION OF THE PHRENIC AND CERVICAL SYMPATHETIC NERVES

This was an attempt to reproduce some work done by Cannon, Binger and Fitz ('16). These authors fused in the cat the anterior root of the right phrenic nerve with the right cervical sympathetic cord. Thus, after regeneration had taken place, the impulses over the phrenic would be sent to the superior cervical ganglion. Out of the six cats operated on four showed, after four months, according to these authors, the following symptoms: marked tachycardia, loose movements of the bowels, falling hair, increased excitability and increased metabolism. One cat also showed exophthalmos and respiratory hippus on the operated side and on autopsy the adrenals were found to be three times their normal size. A. Troell performed the same operation on cats and dogs. His animals survived from twenty to one hundred and seventy-five days. None showed the evidences of hyperthyroidism noted by Cannon and his coworkers.

Langley ('04) in testing the union of different kinds of nerve fibers united the peripheral end of the cervical sympathetic to the root of the phrenic on the left side. The lower half of the cervical sympathetic was excised on the opposite side. The paralytic effects were equal on the two sides for about two months after which they gradually diminished on the left side. This animal lived for six months without showing any change in the nictitating membrane, eyelids or pupil in correspondence with respiration, or any changes similar to those reported by Cannon and his coworkers.

I made an end-to-end suture of the central end of the phrenic and anterior end of the cervical sympathetic cord in ten cats and three

rabbits. These animals survived from one to twelve months. In no case was I able to find any of the changes indicative of excessive thyroid secretion. The animals recovered from the operation readily, ate heartily and were apparently normal in every respect. This would seem to indicate that a physiological anastomosis was not formed or, if formed, the stimuli thus carried were not efficient in increasing the output of thyroid secretion to the point of producing the pathological changes accompanying toxic thyroid hyperplasia in man.

## IV. EFFECT OF REMOVAL OF A SECTION OF THE CERVICAL SYMPATHETIC NERVE ON THE THYROID

Missiroli ('08) cut the cervical sympathetic nerve in rabbits and examined the thyroid histologically. He reported an excess of secretion immediately after with a gradual reversion and final atrophy. Wiener and Floresco (quoted from Wilson and Durante, '16) made similar findings.

TABLE 9

*Results after removal of a section of the cervical sympathetic nerve on the right side in cats*

| NUMBER OF CAT | WEIGHT OF THYROIDS | | DURATION OF EXPERIMENTS |
|---|---|---|---|
| | Left | Right | |
| | *grams* | *grams* | *days* |
| 1 | 0.070 | 0.085 | 45 |
| 2 | 0.130 | 0.130 | 14 |
| 3 | 0.180 | 0.150 | 33 |
| 4 | 0.125 | 0.143 | 49 |
| 5 | 0.050 | 0.050 | 65 |
| 6 | 0.150 | 0.155 | 51 |
| 7 | 0.100 | 0.105 | 111 |
| 8 | 0.080 | 0.080 | 96 |
| 9 | 0.090 | 0.075 | 169 |
| Average | 0.108 | 0.108 | |

Manley and Marine ('15) removed a part of the thyroid gland and transplanted a part. They found that the transplant underwent hyperplasia exactly as did the stump of the gland in place. It also reacted in the same manner to iodine. These authors conclude that at least secretory nerves are not essential to the thyroid.

Table 9 represents the results obtained by removing, under aseptic conditions, about 2 cm. of the right cervical sympathetic cord in cats.

The thyroids were removed, weighed and fixed in the usual manner. The time elapsing between the sectioning and the removal of the glands varied from fourteen to one hundred and sixty-nine days. The difference in weight of the two glands was not significant in any one case. On the other hand, the average weight of the glands to which the nerve was cut was almost exactly the same as that of the glands where the nerve was left intact. Histologically there was no difference in the two glands. They showed a similar amount of colloid taking the same degree of staining.

It would seem therefore that in the cat secretory fibers, if there are such fibers present, play no part in the normal mechanism of thyroid secretion.

## CONCLUSIONS

Adult rats kept under hygienic conditions and fed a high protein diet develop hyperplasia of the thyroid gland.

Rats kept under unhygienic conditions develop hyperplasia of the thyroid if given a standard diet of bread and milk.

Unhygienic conditions plus a high protein diet bring about a higher degree of hyperplasia in the adult rat than either factor taken alone.

Young, growing rats kept under hygienic conditions do not develop hyperplasia of the thyroid when given a high protein diet which will induce this condition in adult rats.

Feces either from goitre patients or from goitrous dogs fail to induce any changes in the thyroid of the cat when given by stomach.

The central end of the phrenic nerve when sutured to the peripheral end of the cervical sympathetic either does not form a physiological anastomosis or, if formed, the stimuli thus carried are not efficient in increasing the output of thyroid secretion to the point of producing the pathological changes accompanying Graves' disease in man.

Removal of a section of the cervical sympathetic cord produces no change in the thyroid gland in the cat indicating that secretory fibers, if there are such fibers present, play no part in the normal mechanism of thyroid secretion.

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# STUDIES ON THE RELATIVE PHYSIOLOGICAL VALUE OF SPECTRAL LIGHTS

## I. Apparatus

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### INTRODUCTION

The present paper is concerned primarily with a description of an apparatus by means of which spectral lights of equal radiant energy content can be obtained and comparative work of wide range carried out. Before proceeding to a description of this apparatus it seems best to point out, as briefly as possible, why such an apparatus is essential.

Against much of the work that has been done on the study of the relative physiological value of spectral lights the objection can be raised that sufficiently accurate methods of experimentation and description have not been employed. Speaking more particularly of the work which has been done on the "color vision" of animals other than man, and excluding the early investigations in which colored light was obtained by means of filters, screens, etc., much inaccuracy is found in those in which spectral light is used. By observing the influence of an entire dispersion spectrum on the aggregation of organisms results are obtained which leave much to be desired quantitatively. Also, in much of the work indefinite and inadequate terms of description are used, in that, e.g., one light is said to be brighter than another, as measured by the human eye, or equally bright, etc. This mode of description is purely subjective. Only qualitative facts are learned when a spectrum is viewed as a whole and this method does not enable us to say by how much one light is brighter than another, brightness being a quantitative conception only when measured photometrically.

It is therefore not permissible to compare the "color vision" of an organism to that of the normal human eye or of the color blind eye

following the use of such inadequate methods. In a recent paper Hess (1) attempts to give an objective, quantitative comparison with the human eye, by comparing the influence of different colored lights on lower organisms with the influence of these same lights on the pupillar reflex of man. In this work, however, Hess makes use of a method, in addition to using colored glasses, against which objections may be raised. He varied the absolute intensity of his source, thus changing the luminosity of the colored lights, until a just perceptible change in the size of the pupil was observed. The relative results which he obtained with different colored lights he compared with those obtained on lower organisms. But this is an analytic rather than a synthetic method. Furthermore, it gives rise to physiological (subjective) differences in the apparent relative brightness value of lights of different color (Purkinje phenomenon). To have observed the comparative amount of change in size of the pupil with colored lights of the same intensity would have given more reliable results.

To return to the objections against the descriptive qualitative comparisons of colored lights as judged by the human eye, it is well known how variable such comparisons are likely to be on account of the variable physiological factors involved. In the first place the state of adaptation (light or dark) of the human retina is all important in the judgment of the relative luminosity of lights of different color. In the second place the absolute intensity of the colored lights must be considered, for the part of the spectrum of apparent maximum brightness shifts as the intensity of illumination is changed, being higher in a strong than in a weak spectrum. Luminosity or apparent brightness, as Troland (2) points out, is a psychological variable which cannot be expressed in physical units. It depends upon the absolute value of retinal illumination and upon the general state of sensitivity of the visual system.

But the chief fault which we have to find is the failure on the part of many investigators to take the unequal distribution of energy into consideration. It seems evident, to obtain quantitative results on a purely objective basis which will permit of comparisons being made exclusive of subjective sensations and which will allow a conclusive correlation of facts, that any comparative investigation of the influence of spectral lights must take into consideration this unequal distribution of radiant energy in the spectrum. The quantity of this radiation in the various colored lights should be ascertained, corrections made for the unequal distribution or, what is better still, the lights of

various wave-lengths be made quantitatively equal by equating the radiant energy in them.

It is true that psychologists and physicists are not unanimous regarding the respective merits of photometry (equation of brightness) and of radiomicrometry (equation of radiant power) in the standardization of colored lights. This is due to the fact that they are primarily interested in light as it influences the human eye.

Photometric standardization (heterochromatic photometry) is a method which gives us quantitatively exact information about different colored lights, although as Parsons (3) says

> Photometry is one of the most faulty of physical measurements, chiefly, to use a paradox, because it is in most cases, not really physical, but physiological.

But a word should be said regarding the question of a photometric standardization of colored lights for comparative work on "color vision," and its seeming inadequacy. In the study of human vision it is essential that the brightness value of colored light stimuli be known and that work be done with visual stimuli of different wave-length but of equal brightness (spectral energy at constant luminosity). The use of such equally luminous stimuli for studying the stimulating value of different wave-lengths on lower organisms would give us quantitative results of interest but of questionable value except in so far as we wished to note the effects on other sensitive protoplasm of lights which are "equally bright" to the human eye. But the problem of visibility (to the human eye) is one that requires a measurement of the radiant energy content and a correction for the same. It deals with luminosity at constant spectral energy. In the work of Nutting (4), (5), Thürmel (6), Ives (7) and Nutting (8) we have the results of the study of this "visibility of radiation," which expresses the relative sensibility of the retina to light of different wave-lengths but of equal energy (Nutting (9) ).

Now in man, as Troland (2) says

> To determine the effect of stimulus intensity we may take any constant wave-length and try our experiment with different values of the intensity, or to find the influence of wave-length, we may select a definite intensity and vary the wave-length. In this latter procedure we are obliged to equate the intensities of the qualitatively different lights which we use.

This latter problem, viz., the determination of the influence of wave-length, is the one that we are primarily interested in when we

make a comparative study of the stimulating value of colored lights. We are therefore obliged to equate (either directly, or later by corrections) the radiant energy, in order to have a quantitative unit for the comparison of qualitatively different light, or for the sake of working with objective, not subjective, units of light.

We believe then, to repeat, that in the study of the evolution of color vision, or of the comparative investigation of the stimulating efficiency of spectral lights, it is absolutely essential that the distribution of the energy in the spectrum be considered, whether it is believed that it will affect the results obtained or not. This is just as important as is the necessity of having as pure and saturated lights as possible. Although it has long been recognized that the sensation of color brightness (the indication of the stimulating value to the human eye) must be due to a combination of wave-length and energy, and the curve for the distribution of this energy known (10), even the recent work on the investigation of the relative efficiency of different wave-lengths has, with few exceptions, failed to take this matter into consideration. Loeb and Wasteneys (11), (12), for instance, do not even refer to the possible influence of the unequal distribution of energy in the spectrum on the results which they obtained. This objection, among others, may also be made to the work of Hess (13).

The disregard of this matter is, however, not universal. Hertel in various investigations (14), (15), (16), determined, with a thermopile and galvanometer, the energy content of the lights of different wave-length which he used. Kniep and Minder (17), also using a thermopile and galvanometer, measured the absolute energy in their colored lights, and equated it (see p. 629 ff.). These investigators used filters for obtaining their colored lights, although they attempted to throw out the infra red and ultra violet rays. (See p. 634.) Blaauw (18, see pp. 271 ff.), corrected for the unequal distribution of radiant energy by means of calculations of its amount in the spectrum which he used, and Mast (19) referred to the importance of considering the amount of radiant energy.

In the work of Laurens (20), Day (21) and Gross (22) a real beginning was made in the study of the relative stimulating value of lights of different wave-length but of equal radiant energy content.[1] Comparative psychologists also have recognized the importance of radio-

[1] This work was done at the Harvard Zoölogical Laboratory and the credit for its inception should be given to Prof. G. H. Parker.

metric determinations of lights used in studying the color vision of animals (23), (24), (25) and (26).

Mast (27) in his recent paper clearly recognizes the necessity of considering the amount of radiation in the various spectral lights, and says (p. 489) that,

> To ascertain the relation between wave-length and stimulating efficiency it is . . . . necessary to make correction for the unequal distribution of energy in the spectrum.

Such corrections were made. No objection can be raised against the physical accuracy of this method, but certainly, as Ives (7) points out, the measurement of the intensity of radiation directly at the eye-slit is the ideal one since it eliminates the determination of corrections. This is the actuating motive which prompted the construction of the apparatus which will now be described.

## AN APPARATUS DELIVERING SPECTRAL LIGHTS OF EQUAL RADIANT ENERGY CONTENT[2]

The first problem was to decide upon an instrument with which to measure the radiant energy in the spectral lights. It was our intention, as above outlined, not to obtain data for the construction of the curve representing the distribution of energy in the spectrum, but after having ascertained the energy contained in different parts of the spectrum to equate this so that it had a certain unit value throughout.

For the measurement of radiation or of the radiant energy content of light, there are a number of physical instruments. For articles dealing with the description and application of these the following, among others, may be cited: Coblentz (28), (29), Schmidt (30), Pfund (31), (32), (33), (34), Day (21) and Kunz and Stebbins (35).

We finally decided to use a Hilger thermopile and a Broca galvanometer. The thermopile which we have has a sensitive area of 20

[2] It gives us great pleasure to express our thanks to the following: Prof. H. S. Uhler of the Physical Laboratory, Yale University, for advice and assistance with the spectroscopic part of our apparatus; Prof. A. H. Pfund of the Physical Laboratory, Johns Hopkins University, for advice concerning the radiant energy measuring apparatus; Prof. H. A. Bumstead of the Physical Laboratory, Yale University, for the loan of a radiomicrometer; Prof. J. B. Watson of the Psychological Laboratory, Johns Hopkins University, for advice on general matters of apparatus—spectroscopic and energy measuring; and Prof. Philip E. Browning of the Kent Chemical Laboratory, Yale University, for the generous way in which he has placed a spectrometer at our disposal.

mm. × 1.5 mm. and a resistance of 2.8 ohms. The galvanometer has a resistance of 10.17 ohms, period 10 seconds, deflection in millimeters at 1 meter for 1 micro-amp 415, and deflection in millimeters at 1 meter for 1 micro-volt 40.81. The resistance was reduced by connecting the coils in parallel.

In order to control the sensitivity of this apparatus, readings of the radiation from a 16 c.p. Standard carbon lamp, burning always on 100 V, were taken. By means of the control magnet, the sensitivity of the galvanometer was varied until deflections of 400 mm., scale at 1 meter, were obtained.

In addition to this apparatus we have a Franz Schmidt and Haensch radiomicrometer (Schmidt (30) and Coblentz (29) ) lent to us by Professor Bumstead.

Before proceeding to the description of our final apparatus for obtaining colored lights a brief reference may be made to one which was finished and then discarded for what will be obvious reasons. This apparatus was based on the plan of the light generators used by Laurens, Day and Gross (20), (21), (22). The light from a Nernst glower (220 V.) was passed through a lens, emerging as a parallel beam. To prevent over-heating a circulating water-cell was placed between the glower and the lens. Later this was given up because it lengthened the focal distance, in favor of a blast of compressed air emerging from a wing-tip burner, directed against the front face of the lens. Aside from the objection of cooling the glower and thus reducing the intensity of light, the glower was made to buckle backward, due to the unequal heating on the front and back surfaces. This happened although the lower connecting wire of the glower was suspended in a cup of mercury. The parallel rays of light were directed upon one of the faces of a large 60 degree angle prism-bottle (10.5 cm. by 10.5 cm.) filled with carbon bisulphide and placed at the angle of minimum deviation. The spectrum thus obtained was focussed by a converging lens and bands of light of the desired wave-lengths were obtained by placing a micrometer slit, opened to the appropriate width, at the focal plane of the converging lens.

This apparatus had the advantage of yielding a spectrum of large size and of great brilliancy. But it was found impossible to obtain with it pure and saturated light when the bands were examined with a spectrometer.

Since this large spectrometer was not successful we decided to obtain our spectral lights by passing white light through a Hilger constant

deviation type spectrometer. At first we used the Nernst glower as a source. It was soon found, when we came to measure the radiant energy of the shorter wave-lengths, that more than one such glower was going to be necessary to give a light containing the unit energy content which, from preliminary experiments we had selected as being most satisfactory. By means of this apparatus we were able to obtain eight consecutive colored lights, each 30 wave-lengths broad, extending from 430 $\mu\mu$ to 670 $\mu\mu$ and containing the same amount of energy (see table 1). The glowers were clustered together and so connected by switches, with their ballasts, that any number, from one to five, could be burned at the same time. For the lights of shorter wave-length a mirror was placed behind the glowers so that their reflected image was focussed on the slit of the collimator.

TABLE 1

*Showing the conditions under which the eight lights, each 30 wave-lengths wide and equal in radiant energy content were obtained*

| NUMBER | RANGE OF WAVE-LENGTHS | NUMBER OF NERNST GLOWERS | WIDTH OF SLIT IN TELESCOPE |
|---|---|---|---|
| | | | *mm.* |
| 1 | 430–460 | 5 (+ mirror) | 4.5 |
| 2 | 460–490 | 4 (+ mirror) | 3.7 |
| 3 | 490–520 | 2 | 2.8 |
| 4 | 520–550 | 1 | 2.3 |
| 5 | 550–580 | 1 | 1.9 |
| 6 | 580–610 | 1 | 1.6 |
| 7 | 610–640 | 1 | 1.3 |
| 8 | 640–670 | 1 | 1.0 |

These eight lights were perfectly satisfactory but for the inconvenience occasioned by burning five glowers so closely together and the consequent bending and displacement. We therefore decided to use a 1000 watt gas-filled Mazda lamp, burning on a 110 V city circuit, the variations occasioned by the fluctuations in the intensity of this current being nullified by the large number of determinations of the radiant energy content which were always made. Furthermore we soon came to the conclusion that eight such lights were not all that we desired, for since we wished to study the relative stimulating value of the various wave-lengths in the spectrum, as well as to find the wave-lengths of maximum stimulating efficiency, it was deemed necessary, in order to be able to say, with an approach to accuracy,

where the region of maximum stimulating efficiency was, to work with a smaller number of wave-lengths. But it is difficult, under our conditions, to work with bands of light less than 30 wave-lengths wide. We decided therefore to increase the number of lights, keeping each 30 wave-lengths wide, but, beginning with a band of light extending from 420 $\mu\mu$ to 450 $\mu\mu$, to advance through the spectrum by 10 wave-length steps. The twenty-three lights thus obtained are listed in table 2.

TABLE 2

*A summary of all the conditions necessary for obtaining twenty-three lights, each 30 wave-lengths wide and equal in radiant energy content*

| NUMBER | RANGE OF WAVE-LENGTHS IN $\mu\mu$ | POSITION AT WHICH DRUM MUST BE SET | WIDTH OF SLIT | DISTANCE ON DRUM | RANGE OF WAVE-LENGTHS ON EITHER SIDE OF D LINE | DISTANCE FROM LAMP TO SLIT |
|---|---|---|---|---|---|---|
| | | | *mm.* | *cm.* | | *cm.* |
| 1 | 420–450 | 434.0 | 4.8 | 12.90 | 549–641 | 9.0* |
| 2 | 430–460 | 444.0 | 4.5 | 11.70 | 552–636 | 10.0* |
| 3 | 440–470 | 454.0 | 4.3 | 10.70 | 555–632 | 13.0* |
| 4 | 450–480 | 464.0 | 4.0 | 9.80 | 557–628 | 14.0* |
| 5 | 460–490 | 474.0 | 3.7 | 9.15 | 559–625 | 2.5 |
| 6 | 470–500 | 484.0 | 3.4 | 8.40 | 561–622 | 4.0 |
| 7 | 480–510 | 494.0 | 3.1 | 7.80 | 563–619.5 | 6.0 |
| 8 | 490–520 | 504.0 | 2.8 | 7.20 | 565–617 | 7.5 |
| 9 | 500–530 | 514.0 | 2.6 | 6.70 | 566.5–615 | 9.5 |
| 10 | 510–540 | 524.0 | 2.5 | 6.25 | 568–613 | 11.5 |
| 11 | 520–550 | 534.0 | 2.3 | 5.85 | 569.5–611 | 13.0 |
| 12 | 530–560 | 544.0 | 2.1 | 5.50 | 570.5–609.5 | 14.5 |
| 13 | 540–570 | 554.0 | 2.0 | 5.20 | 571.5–608 | 16.0 |
| 14 | 550–580 | 564.0 | 1.9 | 4.90 | 572.5–607 | 17.5 |
| 15 | 560–590 | 574.5 | 1.8 | 4.60 | 573.5–606 | 19.0 |
| 16 | 570–600 | 584.5 | 1.7 | 4.30 | 574.5–605 | 20.5 |
| 17 | 580–610 | 594.5 | 1.6 | 4.10 | 575.5–604 | 22.5 |
| 18 | 590–620 | 605.0 | 1.5 | 3.90 | 576–603 | 24.5 |
| 19 | 600–630 | 615.0 | 1.4 | 3.60 | 576.5–602 | 26.5 |
| 20 | 610–640 | 625.0 | 1.3 | 3.50 | 577–601.5 | 28.5 |
| 21 | 620–650 | 635.0 | 1.2 | 3.30 | 577.5–601 | 30.5 |
| 22 | 630–660 | 645.0 | 1.1 | 3.10 | 578–600.5 | 32.5 |
| 23 | 640–670 | 655.0 | 1.0 | 2.95 | 578.5–600 | 34.5 |

* With reflector.

The lamp is enclosed in an asbestos lined, blackened box (see fig. 1), through an aperture in the front of which the light emerges to enter the slit *S*, kept at constant width, of the collimator. The back of the

box is open and holes are bored in the top and bottom for ventilation. The light-box and spectrometer, with the exception of the ocular end of the telescope, are surrounded by heavy black curtains, reaching almost to the ceiling of the room. In order to obtain colored lights of the desired number of wave-lengths, diaphragms of appropriate width

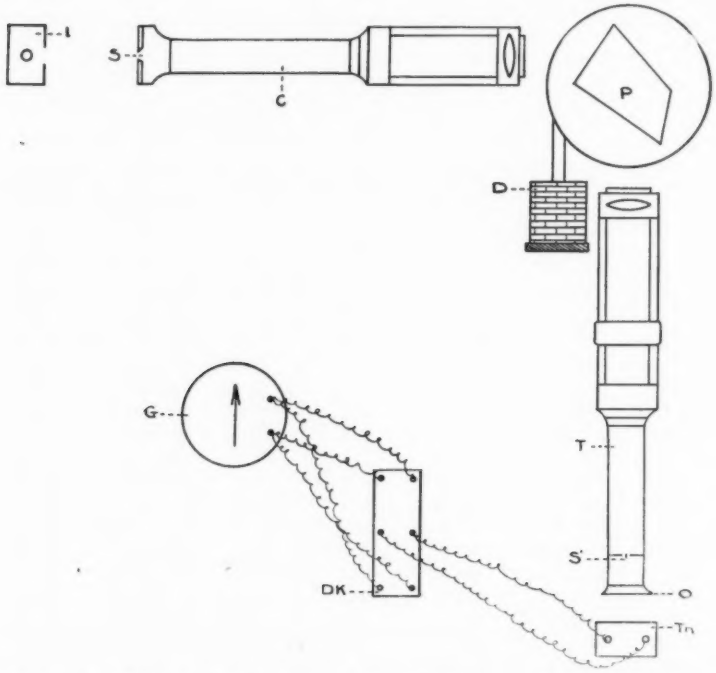


Fig. 1. *L*, lamp in box; *S*, collimator slit; *C*, collimator; *P*, prism; *D*, wave-length drum; *T*, telescope; *S'*, slit in telescope just behind cross-hairs; *O*, ocular; *Th*, thermopile; *DK*, double-key; *G*, galvanometer.

(twenty-three in number, one for each light), are placed at the focal point, directly behind the cross hairs, in the telescope, thus converting the spectrometer into a monochromatic illuminator. These diaphragms consist of cylindrical tubes of blackened cardboard adjusted to fit snugly inside the telescope tube, in the ocular end of each of which a disk of blackened cardboard, with a slit of appropriate width and 8

mm. high is fastened. The width of each slit is such as to allow 30 wave-lengths to pass. As is well known, in the prismatic spectrum the dispersion increases as the wave-length diminishes so that at the violet end a certain number of wave-lengths occupy a wider space than they do at the red end. A wide diaphragm is therefore necessary at the violet end and a narrow one at the red end to let through the same number of wave-lengths. The correct width was determined with the aid of the sodium line as follows: Given a desired range of wave-lengths, e.g., 460 $\mu\mu$ to 490 $\mu\mu$ (see no. 5, table 2); the distance on the wave-length drum between the scale marks 460 and 490 was first measured with a strip of paper and the middle point determined. (This middle point, in this case 474, gives the position at which the drum must be set in order that the desired wave-lengths, 460 $\mu\mu$ to 490 $\mu\mu$, pass). The middle point of the measuring strip of paper was then placed at 589 on the drum and an equal distance measured off on each side of this, thus determining the range of the wave-lengths on either side of the D line, in this case 559 to 625. The diaphragm, with a slit of arbitrary width, was then placed in position in the telescope and the points noted on the drum at which the D line (sodium flame) disappeared when the drum was rotated. The width of the diaphragm slit was then adjusted so that the D line just disappeared when the indicator on the drum pointed to 559 and 625 respectively. Since equal revolutions of the wave-length drum rotate the prism through equal angles this procedure assures that light ranging from 460 $\mu\mu$ to 490 $\mu\mu$ will pass when the diaphragm is in place and the drum set at 474. The width of the diaphragm slit was thus determined for each of the twenty-three lights.

Having procured the colored lights the next step was to equate the radiant energy in each. This was done by varying the distance between the lamp and the collimator slit of the spectrometer until the desired deflection of the galvanometer was obtained. A direct proportionality was found to exist between the bands, as indicated by their middle points, and this distance (see table 2).

The intensity of the first four lights was increased by placing a spherical reflector, obtained from the Spencer Lens Company, behind the lamp. The spectrometer was placed so that the collimator slit was at a distance of 9 cm. from the lamp, and the reflector adjusted so that the reflected image of the lamp filament was focussed on the slit. At this distance a radiant energy content equal to the amount chosen as our unit value was obtained for light no. 1 (420 $\mu\mu$ to 450 $\mu\mu$).

The radiant energy of the next three lights was then equated by moving the spectrometer away from the lamp, without disturbing the position of the reflector.

Table 2 gives a complete summary of all the conditions necessary for duplicating our apparatus and verifying our measurements. The range of wave-lengths for each light, the point at which the wave-length drum must be set, the width of the diaphragm slit in the telescope, the distance measured on the drum between the end-points of the range of wave-lengths, the range of the wave-lengths in question on either side of the D line used in determining the width of the slit, and the distance between the lamp and the slit of the collimator.

## CONCLUSION

It seems of sufficient interest to restate briefly the advantages of the apparatus which we have described.

That it is perfect in the direction for which we have been arguing is not contended, but it certainly represents a distinct advance over anything that has been described for the comparative study of the relative physiological value of colored light in an equal energy spectrum. The degree of error involved is, in our opinion, of much smaller magnitude than the relative degree of sensitivity in the physiological work contemplated.

By means of the apparatus, in the first place, a series of brilliant colored lights of high purity and saturation are obtained, each of which is a band including a fairly small number of wave-lengths, the same for all, and equal in radiant energy content. Therefore no corrections are necessary for this factor. Furthermore, the entire apparatus is easily adjusted and any light obtained with ease. By it an exact specification of the intensity of the various spectral lights is given, the experimental conditions are rendered reproducible and an exact repetition of the experiments made possible.

We are at present engaged in a series of investigations dealing with the relative stimulating values of these lights on lower organisms and on the eyes of higher forms, as well as the study of chlorophyll assimilation and decomposition. But there are various causes which make the completion of any but a small part of this work in the immediate future doubtful. Owing to the conditions occasioned by the great war it is impossible to obtain a spectrometer from England. The spectrometer which we are at present using is borrowed from the chemical

department of the College and we have the unrestricted use of it only during the vacations, thus retarding our progress. Furthermore extra work which we have both agreed to undertake during the coming college term will leave us little time for research.

Since it is highly improbable, therefore, that we can publish anything in the near future concerning results obtained with our apparatus, we are desirous of publishing a preliminary description of it so as to afford those who are interested in similar studies the benefit of our experience in the construction of an apparatus fulfilling what we consider the necessary physical conditions for the study of the relative stimulating value of spectral lights.

## SUMMARY

The reasons are stated why the study of the relative stimulating value of different colored lights should be carried out with a high degree of exactness for all, even the lowest forms. This involves the use of pure "monochromatic" lights, directly measurable in terms of energy and preferably made equal in radiant energy content, thus doing away with the necessity of making corrections for this.

An apparatus is described which delivers lights of equal radiant energy content. Each light is a band 30 wave-lengths wide. The lights now obtained are twenty-three in number and extend, by steps of 10 wave-lengths, from 420 $\mu\mu$ to 670 $\mu\mu$. The apparatus used for equating the radiant energy content in the different lights is also described.

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# THE ALL-OR-NONE PRINCIPLE IN GRADED RESPONSE OF SKELETAL MUSCLE

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The amount of energy transformed in muscle is characteristically far greater than that manifest in the process necessary to arouse the transformation. Passage from stimulus to response involves a distinct breach of continuity. A change, discrete and transitory, precedes a change revealing sources of energy relatively vast, governed in duration and intensity by no conceivable presiding extension of the original excitatory force; and yet, in skeletal muscle particularly, response is docile to behest of stimulus from its readiness to fulfil the requirements of instrumental calibration up to the marvel of emotional expression.

The determination of the general type of mechanism involved in the factor of control is plainly fundamental to the whole conception of the energetics of muscle. That a minimal stimulus should be capable of awakening the massive charge of an unstable system, and at once restoring equilibrium in relative accordance with its own subtle measure of intensity, is a conception fraught with an assumption of organic complexity unique in its nature, and one which places the problem of muscle contraction in a hazardous field. If, on the contrary, the mechanism of control be regarded as discontinuous with the responding system, conceding to the latter an inherent, determinate capacity for energy release, it is possible to reinstate the control factor as a mechanism for simply utilizing multiples of energy yielding elements. The excitatory process, in accordance with its access to one or more systems of definite dynamic capacity, will then discharge one or more in strict relation to that access; that is, in strict relation to its own demands.

The alternatives present so great a divergence, on the score of both nature and complexity of mechanism, that the acceptance of one or the other must determine widely different interpretations of contractile phenomena—gross, microscopic and physico-chemical. For in one case we must recognize the events in sequence as follows:

Excitation → initiation of discharge in responding system → limitation of magnitude of discharge (massive) in strict conformity to magnitude of excitation (relatively meagre); and in the other,

Excitation → full discharge in every responding system to which the excitatory influence has effective access.

Thus the first alternative involves, in the same system, initiative plus limitation; the second, initiative without limitation in all systems excited. In the first, control necessitates an inhibition of discharge, quantitatively selective, in a disturbed unstable system; in the second, it requires only that excitation penetrate to each quantum of energy needed in the response.

The term *quantum* is here used in its non-technical sense. One is impressed, however, with the interesting analogy between the conception of all-or-none units in graded response and the quantum hypothesis[1] originated by Planck. This hypothesis, which in one of its phases applies to the discontinuous absorption of radiant energy by atoms, can be as yet of hardly more than tentative interest to the physiologist; but it is markedly suggestive. In it we picture the continuous radiant stream presenting itself for transformation and the absorbing atoms attaining perforce an elemental period of vibration before assuming their charge. Thus the gradient of absorption proceeds by a series of steps, each of a determinate energy equivalent, the quantum.

On the basis of so strong an analogy it would here seem permissible to adopt the term in a modified technical sense; and, regarding the effective energy content of a biological system discharging always to full capacity as a physiological quantum, I purpose to use the derivative *quantal* to express both substantively and adjectively the conception of structural carriers of such integers of energy in effects discontinuously graded. Although the heart musculature is all-or-none in action and thus itself quantal in a unit sense, skeletal muscle on the other hand is one of the most perfectly graded of reacting tissues; yet even the gross activity of the latter may, on the assumption of the second alternative discussed above, be described by the same term in a multiple sense. A quantal, therefore, may be any all-or-none or, as Verworn (3) has aptly termed it, *isobolic* system. A series of responsive values would be quantal when composed of discontinuous steps

[1] Bayliss has included in his *Principles of General Physiology* (1) a brief discussion of the Theory of Quanta under the chapter on Energetics, with reference to sources. See also Northrup's *Laws of Physical Science* (2) for a compact statement of the hypothesis in a somewhat wider application.

depending upon the additive discharge of successive quantals. If this prove to be descriptive of the responses of a skeletal muscle, the muscle as a whole will then obey a quantal principle although as a whole it notoriously violates the all-or-none law.

The hypothesis of quantal gradation in the response of skeletal muscle has, in fact, been so well supported that little doubt should remain as to its validity as an established theory. Gotch (4) in 1902, presented results indicating

> that as regards both muscle and nerve, the obvious gradation in the intensity of active effect dependent upon intensity of stimulus is mainly due to these tissues being composed of a large number of elements having no physiological continuity with each other.

Subsequently Keith Lucas (5) succeeded in stimulating directly, and later through nerve fibers, a sufficiently small number of muscle elements to reveal complete discontinuity of twitch-response to graded stimuli; and it is to these experiments that we owe the firm basis attained for the conception. Perhaps the most distinct results confirmatory of the work of Lucas are to be found in the course of two papers; one by Symes and Velie (6), published in 1911, and the other by Mines (7) in 1913, in which tracings obtained from the excitation of sartorius nerve filaments functionally reduced in number by various methods show a limited series of abrupt steps.

The discontinuous response of reduced fibral aggregates to relatively continuous change of stimulus intensity is thus the chief evidence for the all-or-none theory of the contraction of the muscle fiber. Although it is conceivable that the fiber itself might be discontinuously graded, in which case the fibrilla or eventually the sarcomere would stand as the insulated quantal, the results of Lucas are to be emphasized as distinctly against such a conception; for by determining the number of responsive elements in each preparation it was shown by him that the steps in the discontinuous gradient were always less in number than the muscle or nerve fibers present. The data obtained by Lucas therefore clearly justify his view that

> The 'submaximal' contraction of a skeletal muscle is the maximal contraction of less than all its fibres.

The conception is so fundamental that effort should not be spared to bring direct and readily repeated evidence to bear upon it. Stiles (8) has very clearly outlined some of the far-reaching implications in-

volved in an acceptance of the all-or-none principle for the single muscle fiber and with Barbour (9) has described phenomena to which it is peculiarly applicable. Reports of investigations are frequently encountered in which important conclusions avowedly hinge upon such acceptance. Verworn (10) has even predicted that the muscle fiber, if isobolic, must also be found incapable of summation in the sense commonly understood. It is therefore unfortunate that a mass of confirmatory evidence does not exist, capable of being without question utilized in interpreting results of experiments on gross contraction, as well as in helping to denote the path of inquiry into the physico-chemical processes involved in fibrillar shortening.

It was the need of thus assuming one or the other of the two views of graded muscle function, in interpreting certain fatigue phenomena, that led me several years ago to attempt a study of localized response in skeletal muscle, disregarding for the time the suggestive bearing of the principle of all-or-none nerve fiber activity, foreshadowed by Gotch and in recent years so convincingly supported, especially by Adrian (11). The problem of method was to observe directly and if possible to record the response of muscle fibers, single or in distinguishable groups, in the hope of obtaining data leading to similarly direct conclusions as to the jurisdiction of the all-or-none law, if such be found to obtain. Cumulative results by various methods, the most significant of which was described in a recent number of this Journal (12), have shown with consistency that magnitude of quick response in skeletal muscle is independent of magnitude of stimulus except in so far as discrete quantities of effect suddenly appear or disappear at appropriate levels of a graded excitatory influence. A fact of related import has also been established; magnitude of uniformly induced contraction in the fiber is constant through appreciable ranges of threshold value. I intend in this communication to present only such experimental evidence as may form a basis for certain theoretical discussions best treated together, reserving extended treatment of methods and results for separate publication under the particular heads.

This paper now concerns itself with the question of the quantal nature of contraction gradients in skeletal muscle, including thereunder first, the gradient of response to graded stimuli; second, the fatigue gradient; and third, the staircase gradient. I am indebted to Prof. A. Wilmer Duff for bringing to my attention, a number of years ago, the analogy between energy quanta and all-or-none effects in response; and to Mr. John P. Eisenberger for furthering by many ingenious devices and patient observations the course of these studies.

## GENERAL METHODS

With few exceptions the experiments have utilized the sartorius of the frog, usually uncurarized. In most cases tetanizing induced currents of brief duration have been employed as stimuli. For the earlier work the muscle was excised and placed horizontally, with its electrodes, in a small moist-chamber carried on a mechanical stage. The electrodes were of copper or platinum, the muscle resting on an indifferent plate and subject above to the light contact of an active needle, microscopically sharpened—the micro-electrode. The illuminated preparation being brought into the field of the microscope, excursions of a fine particle of charcoal powder resting upon the responding region of the muscle were observed over the scale of an ocular micrometer. Tetanizing shocks lasting long enough to afford accurate reading of the scale were applied in graded or uniform sequence by means of an inductorium connected to a Daniell cell, with the secondary coil moving over a millimeter scale by rack and pinion. The primary current was interrupted by an automatic platinum-mercury key. In some cases the muscle was under tension from a delicate spring; more often it was maintained under "isometric" conditions.

Later work has been done under circumstances designed with four purposes in view: first, to cause excitation of minute areas with a minimum of polarization or of tissue injury; second, to permit of rapid, delicate and, if desired, continuous variation of the intensity of the induced current; third, to afford a method of graphic record; and fourth, to maintain the preparation under conditions as nearly normal as possible.

*Excitation.* The essential features of the capillary pore electrode have been described in the paper already alluded to (12). By its use in various modifications it has proved possible to replace metallic electrodes by terminals of a physiological solution, the active member of which is in contact with the tissue over a circular area of a few microns diameter, distinctly less than that of a muscle fiber. The stimulating circuit carries an undulatory current induced in two opposed coils which are adjustable with relation to a bar-magnet rotated by water pressure. One of these coils is geared to a large wheel which, in actuating it, also registers the movement on a greatly magnified scale empirically graduated in millimeters of coil position. This wheel is under complete control by the operator at the microscope. The capacity for yielding graded excitations has been carefully ascertained, in this and

other stimulating devices employed, by application to a muscle or nerve-muscle preparation. That the course of excitation should be graded has been the only demand made upon the apparatus other than such demands as have been involved in the choice of electrodes. It must be understood that all the electrodes employed, although apparently capable of exciting an area within the diameter of a single fiber, nevertheless must stimulate adjoining elements also when either excitation or irritability is sufficiently increased. Additive effects will then occur, and successive quantal steps must be attributed to the spatial widening of the effective excitatory field.

*Recording devices.* No method of recording fiber excursions has, from the observer's standpoint, proved more satisfactory than that of direct reading of the extent of brief tetani on the ocular scale. This has at times been supplemented by what must be regarded as a modification of the excellent method devised by Spaeth (13), who has recorded the movement of melanophore granules by actuating a kymographic writing-point with the rotary adjustment of a mechanical ocular micrometer. The modification adopted consists in omitting the micrometer and employing as an index the margin of a camera-lucida image, the image-forming object (a strip of white paper) being moved over a dark ground in connection with a magnifying writing-lever system. This has the advantage of absolute mechanical detachment from the microscope, with consequent elimination of the slightest derangement from jar; but the disadvantage of requiring a head-rest for uniform alignment of vision. Moreover the general method, necessitating as it does a motor reaction in addition to the sensory recognition of position, introduces, for this particular work, errors which can be compensated only by comparing the frequency range of variates with that of the normal curve of error. The procedure would, however, have been of great service in the absence of the automatic recording method finally evolved. This consists in the registration upon a photographic plate moved by clockwork over the microscope, of the path of a brilliantly illuminated particle of mercury in the form either of a cylinder enclosed in a segment of capillary tubing or of a spherule sprayed under high pressure from a fine nozzle. Finally it has been found possible, with such illuminated points, to read directly on the ocular scale the extent of rapid twitches, since the positive after-image persists long enough to serve as an index of excursion.

*Preparation.* In most of the later work the muscle lies *in situ* with intact circulation. Distinct advantages are thus gained. Aside from

the absence of injury, other than from the removal of the overlying skin or occasionally of neighboring muscles, there is the benefit of having the tissue held at normal tension so that fibers when excited will alter their shape against the highly elastic matrix in which they are embedded, being returned to a state of rest in a position determined by the tension of the mass. Moreover, the possibility of maintaining the blood and lymph vascular connections in active function has proved of great service in minimizing fatigue and thus lengthening greatly the duration of an experiment. Where it has been advisable to moisten or immerse the exposed surface, no difference in result has been noticed as between the use of Ringer's and sodium chloride solution; the latter is now used for this purpose as well as for the pore-electrode. The superficial layer of sartorius fibers is readily resolved under low magnifications, and the recognition of contracting elements is greatly facilitated by the outlines of the blood-filled capillaries traversing them. As the electrode is applied to a nerve-free region of the muscle, the use of curare has proved unnecessary. If the position of the indifferent pole is such that the stimulating current is not led through the tissue mass (a condition attained in the pore-electrode) evidence of nerve response is rarely seen in any region stimulated. This fact holds good for surviving mammalian muscle, upon which a few confirmatory observations have been made.

*Response.* The response is in most cases "isometric," the designation here being purely relative in significance; for the greatest excursion of fiber material is near, and in a direction away from, the extremities, showing that the terminal attachments yield readily to the localized stress; fine linear deformation of a tendon is readily observed. Every contracting fiber carries its passive neighbors with it, but in rapidly diminishing degree as to distance. The linear region of activity is readily noted if the transverse vessels which follow its movement are well injected. The movement actually recorded is of course that of distortion of the delicate fascia overlying the fibers. The superficial fibers of the sartorius are the "slow" fibers, rich in sarcoplasm, as figured by Zenneck (14) after Grützner. Experiment has shown no difference in quantal behavior between these and the fibers of the dorsal surface. It should be emphasized that all observations here considered are with reference to the prompt, rapid activity of fibers and not to late or sluggish movement attributable to tonus, etc. The latter phenomena, especially with reference to tetanus, fatigue and staircase invite separate investigation by this method. The ultimate value of

the method must depend upon its adaptation to absolute quantitative determinations. It is perhaps not too much to expect that through its use the measurement of fiber activity may become an accomplished procedure.

## THE GRADIENT OF RESPONSE TO GRADED STIMULI

By this is to be understood the course of that series of dynamic variables induced in a muscle by varying the intensity of stimulation in a direction from minimal to maximal, or the reverse. The question at issue is whether this gradient is continuous or discontinuous with reference to that of the stimuli producing it.

The study of twitch in fibers has only recently been undertaken. The long series of observations on local tetani required direct confirmation of the principle of Keith Lucas for simple contractions, and the development of the method has finally enabled a series of experiments to be carried out. A graphic result is shown in figure 1, details relating to this and other figures being incorporated in the legends. Reading from right to left, it will be observed that the successive images of the mercury globule are at length surmounted by a fine line, nearly vertical, which is the path of the point of greatest intensity of the light-reflex. This line is identical for rising strengths of stimulus up to a point where a new and higher level is reached by a single contraction, which in turn yields to a third step that now maintains itself even to identity of the sinuous tip of the path of excursion. As stimuli decrease in strength the third level gives way to the first, not passing through the intermediate step seen in the upward gradient of response. The position of the secondary coil was throughout in or near the critical region where it begins to enclose the primary. The movement of the coil after each stimulation was 1 mm., and was produced by means of a very smoothly working rack-and-pinion. The stimulus was therefore without question coarsely graded. By contrast the uniform levels of response are impressive.

Figure 2 presents a series of relative contraction values obtained from direct readings of the excursions of a locally stimulated region of surviving mammalian muscle (diaphragm of woodchuck[2]). The method

[2] The fact of the high persistence of irritability in the tissues of hibernating animals favors the use of this species, *Marmota monax*. That the somewhat inadvertent choice of the diaphragm was fortunate, was shown shortly afterward in Lee, Guenther and Meleney's (15) announcement of the tenacity of function in diaphragm muscle in general. It is a coincidence also that these authors had employed in certain experiments a metallic electrode similar in principle to that used in this instance.

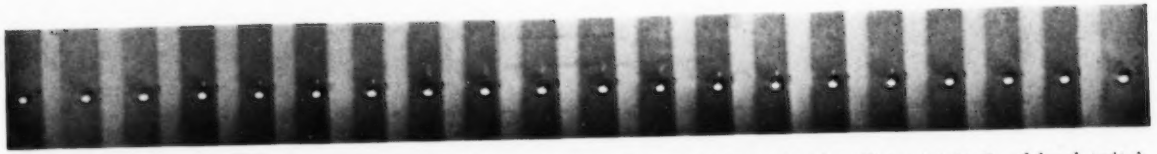

Fig. 1. Experiment 61; July 20, 1917. Photomicrographic record, original magnification (Zeiss AA × 3), of local twitch responses at intervals of 30 seconds in a sartorius muscle (R. pipiens) subjected to break shocks given at successive 1 mm. positions of a secondary coil; reading from *right to left*. The coil positions, with respect to full strength at 0, range from 80 to 70, then 70 to 78, all inclusive. The secondary coil began to cover the primary at 73. The photographic plate was at rest for each stimulation, the abscissa of white circles being successive positions of the illuminated mercury globule at rest. The fine line at length surmounting these areas is the path of excursion. The primary current was furnished by one Daniell cell, with resistance interposed to abolish visible spark at a copper-mercury key. The active electrode was a capillary pore of 7 $\mu$ diameter. The preparation was *in situ*, in communication with the circulation, and immersed in the saline solution which here also served as the indifferent electrode of the non-polarizable circuit.

is in general representative of the earlier work. Brief tetanic contractions were obtained which, with relation to the curve of secondary coil position, are distinctly discontinuous. Steps formed by successive contractions show the same uniformity evident in figure 1, despite crudeness of stimulation method. The series should also be compared with

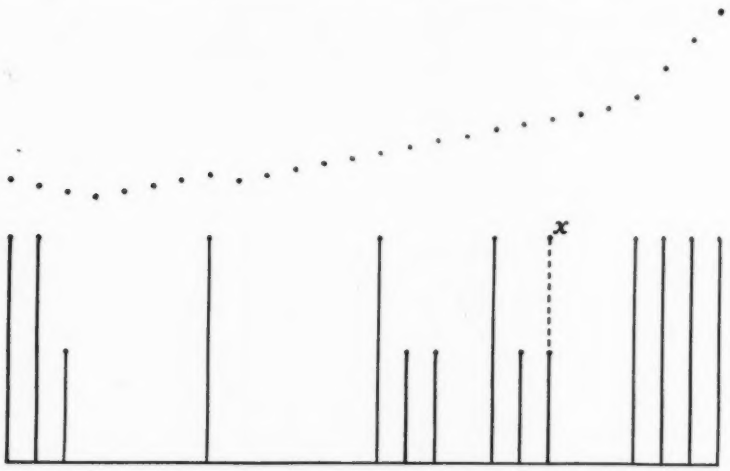


Fig. 2. Experiment 17; April 6, 1916. Plotted from micrometer readings on the application of brief tetanizing stimuli (about 1 second), at intervals of 30 seconds, to an excised portion of the diaphragm of a freshly killed woodchuck, *Marmota monax*. The upper series of relative values are of secondary coil approach; the least difference being 1 mm. A dot denoting a stimulus given at a certain coil position is accompanied, if response occur, by a vertical line below, which shows the relative excursion of a carbon particle on the active region of the muscle. At $x$ the response involved two movements; the first, to the minimal level, followed at once by a further excursion (broken line) to the higher step. A platinum micro-electrode was here used, and the faradic current was produced automatically with a platinum-mercury contact. Coil position is so ranged that a rise indicates rising stimulus, and *vice versa*.

that in figure 3, obtained from the tibialis anticus of the guinea pig by a method of much greater refinement.

The principle here apparent is illustrated diagrammatically in figure 6. The abscissa, $O,O$, the linear measure of time, is at zero level for all quantities involved; these vary in accordance with ordinate distance. The series of stimuli, which varies continuously, intersects the

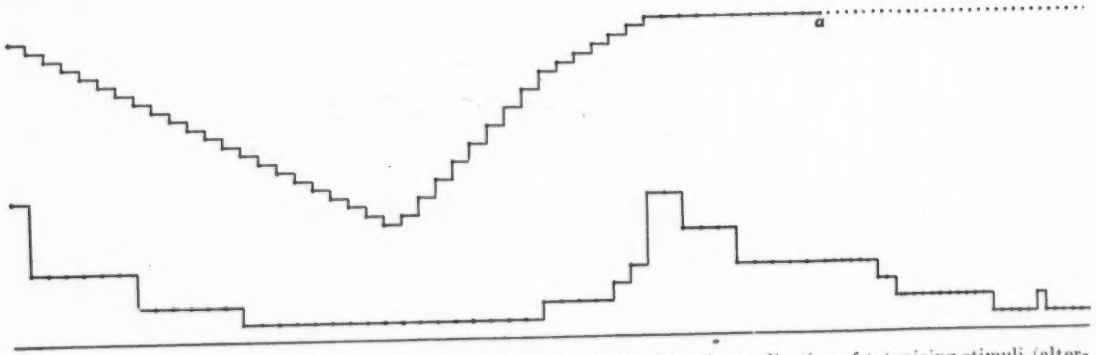


Fig. 3. Experiment 54; June 22, 1917. Plotted from results obtained by the application of tetanizing stimuli (alternating current) lasting 1 second, at intervals of 30 seconds, to the excised tibialis anticus of a freshly decapitated guinea pig. The upper series of points marks the successive positions of the grading coil, with reference to zero position nearest the magnet (least value 5 mm.). The lower series denotes the relative excursions over the ocular scale of the edge of an air-bell on the surface of the muscle. For clearness of comparison the points of each series are joined by lines to show the steps of discontinuity. The coil positions of the magneto-inductor begin at 10 mm., proceed downward to 115; then rise, with partly coarser gradation, to 0, where uniform stimuli are now given at the same interval up to *a*, where the interval is halved for the remainder of the experiment, as indicated by omitting the junction lines. A capillary pore electrode was used (pore diameter 8 $\mu$) and the preparation was immersed in saline solution. The magnet, driven by a constant water-column, was given sufficient uniform speed to cause fused contractions.

uniform threshold, $T,T$, of a single quantal at the liminal point, $L$. At this instant the quantal mechanism is free to act, its energy value rising to $Q$ and maintaining itself as long as stimulus exceeds threshold. Latency, and periods occupied by evolution or regression of energy, are here disregarded; the point $Q$ thus correlates the instant of freedom to discharge with the amount of eventual energy release. Read from left to right, $Q$ marks a quantal step in the ascending gradient of response; read from right to left, it marks a step in the descending gradient.

It is often extremely difficult to determine how many fibers are involved in a given quantal effect. In many cases, however, the minimal response is accompanied by a linear distortion of structure, already mentioned, pointing definitely to the activity of a single fiber, and this activity is found to be irreducible. At other times several fibers appear to be responsible for the minimal effect until the true minimal is differentiated[3] through the eliminative process which will be considered in connection with the fatigue gradient. The contraction abscissa is remarkably stable, though alterations in level occasionally occur. An experiment is seldom complicated by effects attributable to tonus changes if stimulation is not too frequent or prolonged. It is significant that while contractions are proceeding in a given quantal field the stimulus may vary within limits such as are set by fluctuations of current from unequal sparking at a platinum-mercury gap. In fact, earlier results were obtained, as in figure 2, with interrupter and electrodes sufficiently crude to render smooth gradations with a nerve-muscle preparation impossible; yet definite quantal series were produced almost as readily as with the interrupterless magneto-inductor in present use.

There is, in fact, a certain value in results yielded through crude methods of stimulation, which has to do with an objection that can arise as to the validity of the all-or-none tenet. Where stimuli are accurately graded in abrupt increments or decrements, as in figure 1, it could be questioned whether the transition from the subminimal to the apparent absolute "minimal-maximal" effect is not in reality to be ascribed to a change from subminimal to *maximal* in what might reveal itself as a graded system, if intermediate stimuli were applied. The occurrence of the extra step in the upward gradient of figure 1 could be taken as presumptive evidence of this. And deliberately applied series of more

[3] Results obtained with methods for differentiating the activity of single fibers will be published shortly by J. P. Eisenberger.

closely graded stimuli, if at all discontinuous—as must always be the case in the production of twitches—cannot dispose of the same objection; for it might be assumed that the total range of continuous variation in fibral shortening is covered in a shorter span of stimulation strength than can be experimentally applied. Nor can the definite steps in muscle response induced, as in Symes and Veley's (6) experiments, by the diffusion of an anaesthetic into or out of the nerve be rigidly applied as a negation of this objection; for working from the nerve we can be sure only, as Mines (7) pointed out, that certain muscle fibers are *maximally excited*, and not that they are incapable of submaximal response were the nerve fiber in turn capable of graded activity.

Graded stimulation of a nature within limits crude (as in the use of sparking or sliding contacts, polarizable or decomposable electrodes, etc.) introduces, however, a range of adventitious effect for each current step chosen. If a contractile cell to which such stimuli are applied be graded, the adventitious stimuli for each position, for example, of a secondary coil must be reflected in at least some erratic responses. This is not here the case. The response may vary out of grade, as in figure 2, but the variates are determinate and limited. It is impossible to reproduce in either tetanus or twitch of small groups of fibers the turbulence of a muscle submaximally stimulated by ordinary tetanizing currents or by an uneven series of single shocks.

The same difficulty may be attacked through another method, originally devised for the purpose. This method, the basis of which has been outlined (page 521), utilizes an induced alternating current of very low intensity, and a mechanical device whereby a coil is without the slightest jar carried toward or away from a rotating permanent magnet by the turning of a large wheel. Gradation of tetanizing stimuli may thus be made continuous in either direction. Photographic tracings of fiber response obtained with this device were appended to the description of the pore-electrode (12), as illustrative of the method. Two of these[4] are now reproduced on an enlarged scale in figures 4 and 5. Although this form of stimulation applied to a nerve-muscle preparation yields a continuous rising or falling curve of response, it is quite incapable of eliciting such effects when applied locally to fibers. In figure 4 it will be seen that the localized movement, after showing two uniform

[4] Certain details essential to the present discussion were unfortunately marred during reproduction in the former paper. The curves here shown are from the identical prints there used.

twitches in the threshold region, assumes abruptly the tetanic level and maintains it (with a slow upward trend) until the stimulus has again fallen to the threshold, where an almost equally abrupt descent takes place; the gain from the slow tonus-like rise (as yet best unnamed) being then slowly relinquished. Three conclusions may here be drawn: fusion of contractions is a function of the fiber; the process is attended by slow changes; the rise to and fall from the fusion-level are abrupt. The first of these conclusions is patent, while the second requires investigation. The third is here our chief concern. Is this abrupt change of level sufficiently abrupt to denote actual discontinuity of response, or has the contractile substance passed rapidly through all phases of shortening *in graded response to the continuous gradient of stimulus* during the brief periods of rise and fall of the step? It will be necessary to take up another aspect of the problem before considering this question.

## THE FATIGUE GRADIENT

By this is meant the course of that descending series of dynamic varibles induced by repeated uniform excitation.

Fatigue of muscle involves progressive rise of threshold. A stimulus sufficient to produce activity under one set of conditions proves ineffective after a certain amount of work has altered these conditions chemically. It will now be seen that in the light of the all-or-none principle a certain corollary is involved as regards fatigue.

If fatigue imply lowering irritability,[5] it may be assumed that the threshold of a responding element is destined to rise as fatigue progresses. But in an all-or-none or isobolic mechanism the transition from the liminal to the subliminal state is not only critical; it is catastrophic. A range of uniform stimuli while it is, relatively speaking, traversing a progressively narrowing margin of efficacy will, in such a mechanism, still continue to arouse the quantal discharge. The threshold once passed, however, there can be but one result—elimination of the responding factor. Is quantal elimination a characteristic of the fatigue gradient?

A fatigue curve produced by strictly local stimulation is almost always markedly different from that ordinarily secured. Contractility tends to maintenance; irritability to diminution. Even the imposition (for example, at 5 second intervals) of successive tetanic excitations—

[5] The careful determinations of fatigue thresholds by Gruber (16) leave no doubt as to the constancy of this factor.

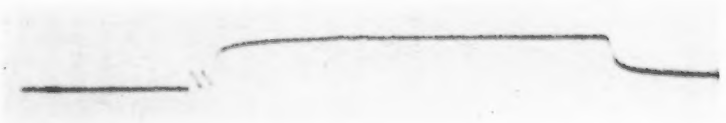

Fig. 4. Experiment 45; February 20, 1917. Photomicrographic record (negative) traced by an illuminated mercury globule resting on the sartorius (R. pipiens). Zeiss AA × 1 (the actual magnification in this figure is approximately 67 diameters, 5.2 times that of the original tracing). The coil of the magneto-inductor, by the mechanical device, was rapidly and continuously approached from the 100 mm. to the 5 mm. position, and at once similarly removed; the entire procedure occupying about 8 seconds. Throughout this time the stimulating circuit was closed and the response meanwhile recorded on the uniformly moving plate. The electrode, with the entire arrangement of apparatus accessory to it, was that described in the previous paper referred to in the text. This and the following tracing read from left to right.

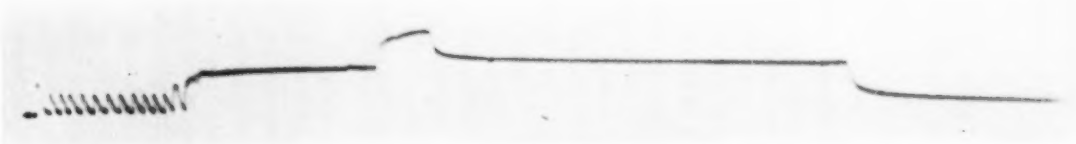

Fig. 5. This tracing is from the same experiment as is that in figure 4, and was made after three tests (similar to those of figure 4) had intervened. Fatigue should therefore be manifest. The experimental conditions are the same, except that the coil, after being brought to the position of maximum stimulation at 0 mm., is then left stationary, so that the second two-thirds of the curve is written during continuous uniform stimulation. The final drop, indicative of rising threshold, is to be carefully compared with that of figure 4, induced by falling stimulus, and their identity of contour noted.

an admittedly fatiguing process—will produce a series of uniform contractions. The threshold, however, if tested is found to diminish; and suddenly contraction disappears, or descends as abruptly to a lower level. This form of fatigue manifestation thus differentiates itself sharply from gradual descents which can sometimes, but not always, be produced by more frequent excitation.

In figure 5 is shown such a quantal elimination. The continuous series of undulatory stimuli rises, bringing about two steps of tetanic contraction increment. The stimuli are then *maintained uniform*. The second step is quickly lost and, after a longer interval, the first also. The general contour of this automatic record should be compared with the plotted results terminating figure 3. The one is obtained from batrachian, the other from mammalian muscle; one produced by continuous, the other by intermittent tetanizing stimulation. The general resemblance is evident.

Two characteristics of figure 5 are now to be noted. The first is the independence of the slow increment, especially at the top of the second step, of the abrupt changes defining the steps. This increment is additive upon the quantal effect, as seen in the descent of the step thus augmented to a new level determined by that increment. The second characteristic lies in the identity of the contour of the final descending step with that of figure 4. The former is an elimination through increment of threshold; the latter, through decrement of stimulus. Both belong to the same fibral region, without change of electrode position. That the result is identical may be seen by superimposing a tracing of one curve upon the other. In view of the magnification (67 diameters) it is difficult to attribute this to coincidence.

The apparent identity of the results of quantal fatigue and quantal decrement of response to falling stimuli furnishes now an additional test to the objection raised in the last section. On the assumption that in the fatigue process there is a continuous increase in the effect of metabolites tending to raise the threshold, the influence of such increase would in a continuous or heterobolic responding system yield a downward gradient correlated with the subsiding irritability. It is hardly possible, however, that such a process could keep pace with that dependent upon a rapid decrement of excitation; much less that the resulting curves should be identical. The conclusion is therefore forced that we are dealing here with a determinate effect, inherent in the responding system, elicited as it is by two widely different conditioning

phenomena. We are working in a realm of self-adjustment where a qualitative test, so to speak, can yield nothing but a quantitative result.

### THE STAIRCASE GRADIENT

This indicates the course of that ascending series of separate dynamic variables induced by repeated uniform excitation.

If the staircase effect in muscle be characterized by progressively lowered threshold, its quantal character may be predicted by derivation from the fatigue corollary. For as the irritability of inactive elements rises, the excitatory influence, traversing the field of inefficacy, must at length span the threshold. The point at which it becomes effective will be critical; and, by the all-or-none principle, the full charge will assert itself. Does the staircase exhibit such quantal accession?

A practically constant phenomenon observed in the course of these studies has been an initial fall of threshold when a previously unstimulated or a rested preparation has begun a series of responses. Moreover, if stimulation has at first elicited a certain contraction step, an additional step may appear while the stimuli are maintained uniform. Persistent excitation will then abolish the second and, eventually, the first step also. These effects have recently been obtained for both twitch and tetanus with every precaution taken for uniformity of stimuli. Following are two series of micrometer readings, in scale divisions, from the sartorius of the frog:

*Fresh preparation; twitches, 1 per second:*

5–5–5–5–12–12–12–12–0–12–12–12–5–5–5–5–0

*Rested preparation; tetani, 1 per second:*

5–5–5–15–25–30–30–0

The quantal conception of staircase and fatigue is set forth in figure 7. It will be seen that this construction is derived from that of figure 6 by simple rotation, clockwise, of the system of lines $S,S$ and $T,T$ about the point of intersection $L$, so that $S,S$ is now horizontal, and thus denotes stimuli of uniform intensity. Read from left to right, the descending threshold $T,T$ intersects the range of stimuli $S,S$ at $L$, the liminal point, thereby releasing the quantal charge $Q$, which manifests itself so long as stimulus exceeds threshold. $Q$ now marks one step in the staircase gradient. Read from right to left, the events are reversed, and $Q$ marks a step in the fatigue gradient. According therefore to the conception here delineated, both fatigue and staircase are to be

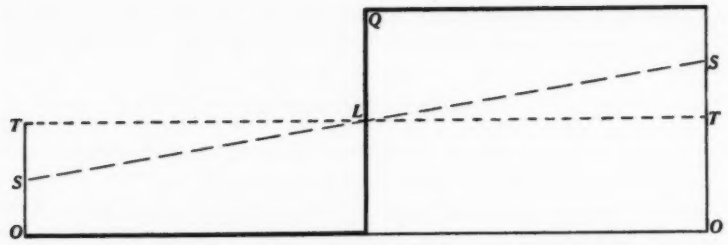


Fig. 6. Diagram to illustrate the mode of unit-quantal release when the stimulus-gradient, $S,S$, intersects the threshold, $T,T$, at the liminal point, $L$. At this point the quantal energy value rises from zero level, $O,O$, to $Q$ if the scheme is read from left to right; or falls from $Q$ to $O,O$ if read in the reverse direction.

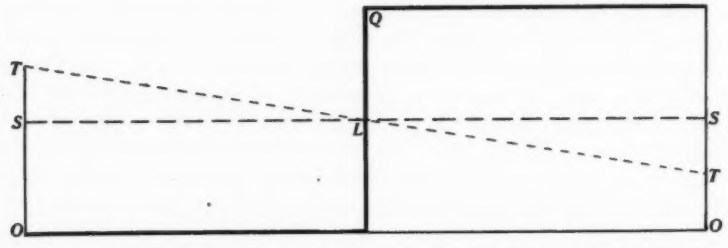


Fig. 7. Rotation of the system of intersecting lines, $S,S$ and $T,T$, to the position shown here, makes the stimulus uniform and the threshold variant. The quantal result is the same.

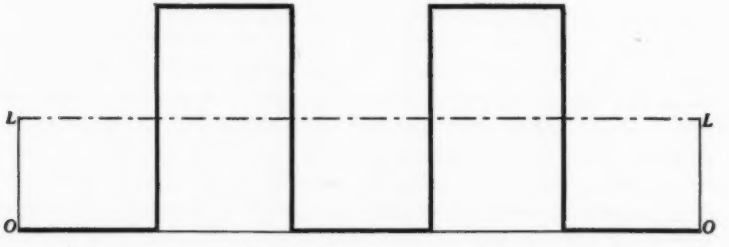


Fig. 8. Here stimuli and threshold coincide along $L,L$, the liminal line. The resulting state of indifference induces quantal fluctuation.

grouped as manifesting a gradient, quantal in nature, of *response to graded threshold*.[6]

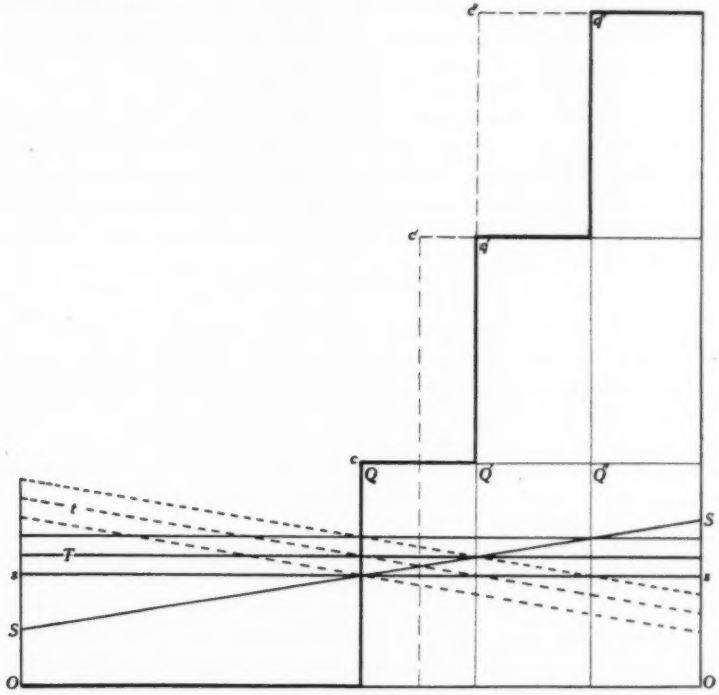


Fig. 9. This diagram combines figures 6 and 7, with two additional thresholds. If the rotation to $t$ of the threshold system, $T$, is made, for each line, upon the vertical of $Q$, ($S$,$S$ now becoming $s$,$s$) the resulting quantal gradient, $Q$,$q'$,$q''$, will remain constant; for the threefold intersection of thresholds with stimulus must precipitate in either position the quantal discharges, $Q$,$Q'$,$Q''$, in sequence, the additive result of which is the step-like gradient of response. If, in rotation of thresholds, the gradient of stimulation, $S$,$S$, retain its position as the range of a variable, the steps of response will here be determined as $c$,$c'$,$c''$. Further interpretation is given in the text.

[6] Compare Verworn's (17) illuminating conception of the basic nature of stimuli. We have here what may be regarded as a constant determining condition, the series of uniform excitations, added to which is a variable factor, the threshold—a function of irritability—the immediate determinant of quantal occurrence. If, as Lee (18) has so clearly demonstrated, fatigue and the treppe are

Fatigue and staircase are not of course to be accounted for wholly, or probably even largely, by change in irritability leading to quantal effect. Observations of localized contraction series have denoted without question that the continuous gradients under uniform stimuli so familiar in the heart are also characteristic of the skeletal fiber; these however, as in the heart, are often absent when the all-or-none effect is readily elicited. The logical necessity of the latter in skeletal muscle if threshold change in fatigue is once admitted (granted the all-or-none principle) is borne out by results. The aim here is to aid in the differentiation of a probable factor in fatigue and its ally—the quantal factor.

## QUANTAL FLUCTUATION

It is obvious that the quantities proper to the lines $S,S$ and $T,T$ of figures 6 and 7 may become errant in both and that the liminal point, $L$, may consequently take any position in a wide field of possible intersection. Yet the dynamic constant of which $Q$ is the symbol must invariably appear or disappear at this point, in accordance with the direction of reading adopted, unless the two gradients happen to coincide. In such instance the possibility of a distinctly different effect must be considered.

During observations on localized contraction there has always appeared a tendency to uniform oscillation, often if not usually rhythmic, whenever stimulus and threshold are in the region of coincidence. Such oscillations are manifest in figures 4 and 5, where they appear to bear an interesting relationship, now under investigation, to the genesis of tetanus in the fiber. The matter of concern here, however, is that the point $L$ in figures 6 and 7 may be regarded as a point of indifference, the slightest departure from which on either of the intersecting gradients must precipitate the quantal effect, positive or negative. If either stimulus or threshold be oscillatory, quantal rhythm may be expected; and simultaneous oscillations could thus result in rhythmic interference effects. Verworn's (19) discussion of the interference of excitations is here suggestive although not directly applicable.

both dependent upon the presence in varying degree of diffusible products, the *variation* therein may be taken as the most delicate form of graded influence possible to apply in emphasizing the discontinuous nature of response.

In figure 8 is shown the diagrammatic result of making the threshold coincide with the series of stimuli. The line of coincidence $L,L$ thus becomes the locus of the liminal point. Indifference is established; and the quantal value tends to fluctuate.

## CONSTRUCTION OF A QUANTAL GRADIENT

If we assume the presence of additional quantals in the constructions of figures 6 and 7, assigning to them thresholds of equidistant value, there will be produced a series of uniform steps identical for both constructions. This is shown in figure 9.

In this diagram, reading from left to right, the line $S,S$ (rising stimuli) intersects the system of lines, $T$ (uniform thresholds), at three liminal points, there determining the quantal discharges, $Q,Q',Q''$ which, by addition of effect produce the series of steps, $Q,q',q''$ (ascending gradient of response to graded stimuli). Similarly, the rotation of the system of thresholds to the position of the dotted lines, $t$, with the stimuli now uniform, as $s,s$, will yield identically the same quantal effect (staircase gradient). Again, reading from right to left, there appear the descending gradient of response to graded stimuli and, by rotation, the fatigue gradient.

Moreover, by taking the system of thresholds in the position $t$, and retaining the stimulus as a variable on the line $S,S$, a new quantal gradient, compositely induced, results; giving the steps $c,c',c''$.

The thresholds being equidistant in value as well as parallel in trend, a line joining the steps of any gradient of response would be a straight line. It is evident, however, that differences in threshold levels and trends, and in the position relative to them of the gradient of excitation, may be introduced to an infinite extent, producing effects seemingly turbulent yet quantally derived. Accessions, eliminations and fluctuations could thus arise, combined and in sequence—functions of the additive and sequent positions of liminal points of intersection and liminal lines of coincidence.

## HYPOTHETICAL DISCUSSION

If muscle be subject to such fluctuant activity of its elements as its quantal nature would seem to necessitate, it would appear requisite to view the effector-periphery as a domain of intensively varying response. The already mentioned observations of Barbour and Stiles (9) on the tetanic shortening of the frog's gastrocnemius are here most sug-

gestive. These authors observed directly the behavior of superficial areas of the muscle during reflex and spinal stimulation and found that tetanic contractions, though apparently of continuously uniform tension, nevertheless involved rapid change of the muscular contour—"swift alternations of activity and rest"—recalling the pseudo-tetanus of Boruttau (20) confirmatory of similar findings by Gad, as well as Lingle's (21) observations on tone in plain muscle. Accurate control by central cells under such conditions must involve an adaptation almost unbelievable in delicacy if the responding factors tend constantly to fluctuate in individual obedience. Just as evidence of this control may be recognized in the alternating play of fibers and fasciculi in the reflexly or centrally stimulated musculature, so may evidence of its absence be found in the well-known tumultuous character of submaximal tetani bereft of central guidance.

At the same time it must be recognized that an all-or-none muscle fiber, actuated by the determinate impulse of an all-or-none nerve fiber, furnishes to the motor cells a servant whose action is, so to speak, *predictable* if it acts at all. It is interesting to consider whether the adoption by the most highly differentiated tissues of what is apparently the simplest device for gradation—the unit system—is not an adaptation best favoring accuracy of control. One is reminded of the similar principle in such diverse, yet not inconceivably related, concepts as those of valence, specificity, the assumption by electrified bodies of unit-charged ions (Millikan) and the transmission and segregation of Mendelian units; as well as the mutations of energy by the quanta of Planck. Verworn (22) has analyzed the conditions under which, in his opinion, an all-or-none or isobolic system (notably the nerve fiber) may in the process of chemical impairment become transformed into a continuous or heterobolic system. The evidence, however, upon which the present argument is based would seem to show that the skeletal muscle fiber adheres with some tenacity to its isobolic régime. Apparently its effective response requires not, as is presumable in more sluggish effectors still subject largely to the more primitive chemical control, an inducing set of variable commands; but solely the unvarying, instant behest. Its response must in turn be instant, determinate, even predictable in consciousness. If it fail, it is in the act eliminated and supplanted.

Gradation of quick response is fibral; not fibrillar or sarcomeric. That is, the fiber as a whole forms the unit employed in effects of multiple constitution. It is to be assumed that in the response of a fiber all of the sarcostyles take part to the full dynamic capacity of each and

every segment. The conception brings the interest of contrast to the inescapable problem of the physical basis and the mechanism of the more deliberate muscular movements, for the origin of which there exist various working hypotheses; and these phenomena can be differentiated only through intensive study of the fiber. For it is obvious that when the additive effect of quickly contracting elements can simulate slow movement or sustainment of the muscular mass, the gross study of tonus, relatively simple in the heart, becomes here a problem in analysis which cannot but lead to baffling interpretations. The absolute conception of the all-or-none principle for the fiber in its response to graded stimuli must, even when the cell is not deleteriously affected, be qualified by the recognition of possible graded activity on the part of systems in the fiber not concerned in quick response (23). The significant observations of Porter (24), on the graded reaction to graded stimuli of tonus factors in the heart, should encourage similar scrutiny of slowly developed movements in the skeletal fiber, the differentiation of which, as already indicated, is hardly possible in the study of the muscle as a whole. For these reasons it is probable that knowledge of the heart is in many respects far in advance of that embodied in the stupendous literature of skeletal muscle.

We are accustomed to state the all-or-none law with a warning qualification: only the stimulus can vary. Alter other, especially chemical, conditions, and the law fails or must be reapplied. It is evident, however, in considering the single fiber of skeletal muscle that alteration of such conditions as are reflected in change of threshold must tend not to relative, but to absolute responsive result, as shown in the precipitation of quantal effect or defect at the crossing of stimulus and threshold gradients. The heart conserves its power by interposing between periods of absolute activity its periods of absolute rest. It is not improbable that, in the fiber of skeletal muscle also, the one phase is conditioned by the other.

## SUMMARY

1. Of the two possible views as to gradation of activity in skeletal muscle—one assuming continuity of response, the other discontinuity—the latter seems the more in accord with simplicity of control; and the term *quantal* (suggested by the concept of energy quanta) is adopted to express briefly the idea of dynamic unit structure in the constitution of contraction gradients. The strong support already attained for the conception, chiefly through the work of Keith Lucas, makes it desirable

that all possible confirmatory evidence be brought forward. The present study aims to apply the principle not only to response following graded stimuli, but also to the gradients of fatigue and staircase effect. (Pages 517 to 520.)

2. The methods employed have ranged from simple means for local stimulation and observation, to various devices especially designed to secure extremely minute application of the stimulus without disturbing factors, delicate and continuous variation of the strength of stimuli, automatic record and normal conditions in the preparation. Recognition of contractile elements in a muscle is dependent upon characteristic structural signs of local distortion. (Pages 521 to 524.)

3. Experimental evidence is presented to confirm, both for twitch and for tetanus, the contention that a continuous gradient of stimulation induces a discontinuous gradient of contraction in skeletal muscle, both amphibian and mammalian. The principle is illustrated in diagrammatic form. (Pages 524 to 528.)

4. The possible objection that graded fiber activity might be missed in the interval between two successive stimuli, even when closely spaced, is met by citation of the quantal results of adventitious stimulation strengths, such as must occur in crude methods of excitation. Similar results from tetanizing stimuli, continuously graded, are also of significance in this connection. (Pages 528 to 530.)

5. That fatigue in skeletal muscle must bear, at least in part, a quantal character is deduced from the necessity for the precipitation of all-or-none effect, if rising threshold be granted as a characteristic of fatigue. Evidence that elimination of contractile elements takes place in fatigue, locally induced, is given; and further evidence that this process is identical with the subsidence of the discontinuous contraction gradient during continuously diminishing stimulation is taken as an added negation of the objection raised above. For rising threshold in fatigue is continuous and prolonged when compared with falling stimulus as experimentally applied. The results being identical, as indicated in the superimposability of two reproduced curves, the determinate nature of the fibral energy system would seem to demand acceptance. (Pages 530 to 533.)

6. The staircase effect, if correlated with falling threshold, must likewise share in the quantal character of its reciprocal phenomenon, fatigue; experimental observations are brought forward confirmatory of this deduction. A diagram is constructed to show that the quantal response in fatigue and staircase may be viewed as a manifestation of

response to graded threshold. The occurrence, however, of continuous gradients in fiber activity, under these circumstances, is not to be questioned; but results would indicate that they are clearly capable of differentiation from quantal elimination and accession. (Pages 533 to 536.)

7. Wherever stimulus and threshold coincide, indifference is established; with precipitation of quantal effect, positive or negative; and hence a tendency to fluctuation, often rhythmic. Diagrammatic illustration is given of the result to be expected when excitation and threshold gradients tend to coincide. (Pages 536 to 537.)

8. A quantal gradient (all-or-none steps) may be constructed by derivation from the diagrams already cited. This construction is illustrated and described, and the complex result pointed out of assuming variable instead of fixed gradients in the functional muscle complex. (Page 537.)

9. The possible significance of the above factors in determining the known fluctuant activity in the tetanized muscle is discussed, especially with reference to the demand for central control. The determinate nature, however, of fiber contraction probably renders central control more accurate than could be the case in a continuous mechanism, based, as that control is, upon a physiological unit-system. In contrast must be considered the character of phenomena related to tonus, readily confused with the results of certain quantal effects in the muscle as a whole, but sharply differentiated in the fiber, as they are in the heart. The quicker movements, all-or-none in nature, would seem to tend, as in the heart, to alternations of work and rest. (Pages 537 to 539.)

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# THE ALLEGED RELATION OF THE EPINEPHRIN SECRETION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPERGLYCEMIAS

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The view has been expressed by various writers that some, perhaps most, of the forms of experimental hyperglycemia (asphyxia (1), piqûre (2), etc.) are dependent primarily upon excitation of the adrenal glands to increased secretion of epinephrin, which then causes an accelerated mobilization of sugar in the same way as adrenalin does when artificially introduced. It is against this idea that subcutaneous injection of adrenalin is more effective in producing hyperglycemia and glycosuria than intravenous injection. However, it has been cited as evidence that the adrenal intervenes as an essential factor in the hyperglycemias in question, that a given experimental hyperglycemia which can be shown to occur while the adrenals are intact cannot be obtained after their removal. Further, some observers have stated that even the normal sugar content of the blood is not maintained after adrenalectomy, hypoglycemia being present; that glycogen cannot be normally stored by the liver, and so on.

The experimental basis for these conclusions seems to us to be quite unsatisfactory. Observations in which the production of hyperglycemia by a given procedure before removal of the adrenals are compared with similar observations after removal of the glands, suffer from two serious defects. In the first place, the animal has been deprived of organs essential to life. The period of survival is very brief in the absence of accessory glands, and when an animal is going to die within twenty-four hours it is surely a matter of difficulty and risk to fix the point up to which such a function as the regulation of the sugar content of the blood may still be regarded as normal. Secondly, the animal has been subjected to a major operation; it has been anesthetized for a time; possibly it has been prepared for the operation by a

period of fasting; certainly it does not eat after the operation. All these things cannot fail to complicate greatly any observations upon changes in the blood sugar dependent upon an increase or diminution of the rate of mobilization of the liver glycogen.

We have put the question of the relation of epinephrin to some of the forms of experimental hyperglycemia to the test by a method which eliminates all these disturbing factors. The epinephrin liberation was abolished, within the limits of sensitiveness of the methods used for its detection, or reduced to an insignificant fraction of its normal amount by dividing in cats the nerve supply of one adrenal and excising the other gland. After recovery from the operation the sugar was estimated from time to time in samples of blood obtained under the various conditions which it was desired to study, these being compared with normal samples previously collected.

## TECHNIQUE

The sugar was estimated by the method of Lewis and Benedict (with Pearce's modification). Blood was collected from a vein, usually the femoral, by puncture with a hypodermic needle attached by a rubber tube to a 2 cc. pipette, in the point of which were a few crystals of potassium oxalate. The skin over the vein was shaved, usually the day before the blood collection, so that the animal might have recovered from the disturbance due to the shaving. Generally both legs were shaved at the same time so that blood could be obtained from either vein. Occasionally the jugular, and once or twice the external saphenous were employed. The needle was pushed directly through the skin into the vein. After the blood samples desired had been collected, a little tincture of iodine was placed on the skin over the site of the puncture, and a considerable interval, usually about a week, was allowed to elapse before any further samples of blood were taken. This refers to the observations in which such procedures as asphyxia and anesthetization had been resorted to. The blood was drawn up into the pipette a little above the mark, the pipette disconnected from the needle and the blood after being allowed to flow back to the mark, immediately discharged into a large test-tube containing 8 cc. of distilled water. The test-tube was then shaken. After hemolysis had occurred 15 cc. of saturated solution of picric acid was added and the contents filtered, after being well shaken. Duplicate quantities (7 cc.) of the filtrate were measured into test-tubes graduated in 0.1 cc. from 1 to 20 cc. Two cubic centimeters of saturated solution of picric acid and 1 cc. of 10 per cent solution of anhydrous sodium carbonate were added to each test-tube. After removal from the autoclave, any loss of fluid was replaced up to 10 cc. After filtration the color was compared with the picramic acid standard recommended by Lewis and Benedict, and also with a solution of dextrose of known percentage which was carried through the same process as the blood. Before deciding to use Pearce's modification, we compared it with the original method and found a sufficiently close agreement, as illustrated by the

following figures obtained with different quantities of a solution of dextrose. By the polarimeter it was estimated that 100 grams of the dextrose used corresponded to 93.6 grams dextrose. From this a solution was made of such a strength that 1 cc. would contain 0.936 mgm. dextrose.

| DEXTROSE SOLUTION | LEWIS AND BENEDICT METHOD | AUTOCLAVE MODIFICATION |
|---|---|---|
| *cc.* | *mgm.* | *mgm.* |
| 1.0 | 0.881 | 0.890 |
| 1.0 | 0.910 | 0.940 |
| 2.0 | 2.060 | 2.012 |
| 0.5 | 0.418 | 0.411 |
| 0.5 | 0.461 | 0.427 |
| 0.75 | 0.748 | 0.741 |

In this paper are reported observations upon two forms of experimental hyperglycemia, that produced by asphyxia and that produced by ether anesthesia. The routine was to obtain a preliminary specimen of "normal" blood and then to subject the animal to a period of asphyxia or anesthesia. Asphyxia was maintained by placing over the nose and mouth of the animal a metal cone covered with a towel. The effect of the asphyxia was controlled throughout the whole period by palpating the heart through the chest wall. When distinct slowing of the heart had been produced the animal was allowed to breathe freely for a few seconds and the asphyxia was then repeated. The object was to produce and maintain with the necessary intervals of free breathing, a distinct asphyxial condition for ten to twenty minutes. The asphyxia was never pushed so far as to endanger life. As regards the anesthesia effect, an ordinary surgical anesthesia was maintained for fifteen to twenty minutes. In a few observations the etherization was less complete, stopping short of disappearance of the corneal reflex. In a number of the experiments when the animal happened to be especially quiet during the collection of the preliminary specimen, it was immediately afterwards subjected to frightening by a dog for twenty minutes to an hour, and another blood specimen then collected. Generally the animal was free in a small cage during the frightening, but in a few observations it was tied on a holder. In no case was it possible for the dog to inflict any physical injury on the cat. In some cases the sugar content of blood specimens, obtained after varying periods during which the animal remained tied down, was compared, although no systematic observations on the so-called "Fesselungs" hyperglycemia were made. The frightening experiments were followed by an asphyxia observation, in order to determine whether the animal was capable of showing a decided hyperglycemia. In this way it was supposed that a negative result due to poverty of the glycogen store of the liver could be taken account of. In a few of the preliminary observations single blood specimens were obtained, either "normal" or during asphyxia or anesthesia. Although the hyperglycemia associated with these conditions can be demonstrated without difficulty by such isolated observations made on different days on the same or on different animals, it is far better to compare a succession of samples taken on the same occasion from one and the same animal.

Control observations were made on normal cats and also on cats on which laparotomy had been performed, with a certain amount of manipulation of the abdominal viscera, in order to imitate the surgical procedure in the adrenal operations, except that the adrenals and their nerve supply were left intact. It was not necessary to estimate the epinephrin output in the control animals, as we have previously shown (3) that epinephrin is invariably present in the adrenal vein blood of normal cats when tested under our experimental conditions, and that the amount liberated per minute in different individuals varies within rather narrow limits. All the animals, those in which the adrenal operation had been performed as well as the controls, were kept on the same diet and housed together (in the open air for a large part of the time). Except when purposely restricted, the diet was such as to favor the accumulation of glycogen in the liver (rice with milk, pig's liver, with fish occasionally). In the observations before July 9, no rice was given. Glycogen in plenty was demonstrated in the liver in animals which were giving off no detectable epinephrin, (e.g., 4.75 and 4.26 per cent. in two operated cats; 1.95, 2.55 and 4.13 per cent. in three control cats).

## EFFECT OF THE OPERATION ON THE OUTPUT OF EPINEPHRIN

Our previous experiments (3) showed to what insignificant proportions the liberation of epinephrin (as determined by the denervated eye reactions without drawing blood, or on rabbit intestine and uterus segments with shed blood) is reduced by the operation practiced, even when it is not completely abolished. The values for the residual liberation in the seven cats used for survival observations in that investigation are displayed in table 1. In five of the cats the output of epinephrin was only $\frac{1}{25}$ to $\frac{1}{170}$ of the average output for normal cats. In two of the seven animals no epinephrin whatever could be detected, although in one of them (cat 52) the intestine and uterus segments were so sensitive that $\frac{1}{1000}$ of the average normal output per kilogram per minute could have been estimated. In the other (cat 46) $\frac{1}{550}$ of the normal output could have been detected by the segments and $\frac{1}{600}$ by the eye reactions, which in this case happened also to be extraordinarily sensitive.

It is out of the question to assume that in this experiment epinephrin was present in the adrenal vein blood in a concentration just below the threshold of detectability for *both* rabbit segment and eye reactions. It is therefore as certain as anything can be which has not been actually demonstrated that if any epinephrin whatever was being given off in this animal, it represented much less than $\frac{1}{600}$ of the normal output.

We might then have assumed with confidence that the cats operated upon in a similar way for the experiments on hyperglycemia would be practically incapable of liberating epinephrin from the adrenals, either under normal circumstances or in conditions which have been supposed to cause increased liberation through the adrenal nerves. Never-

TABLE 1

| NUMBER OF CAT | WEIGHT | EPINEPHRIN OUTPUT | | FRACTION OF NORMAL LIBERATION* | | DAYS AFTER OPERATION | EYE REACTIONS |
|---|---|---|---|---|---|---|---|
| | | Per minute | Per kilogram per minute | Per animal | Per kilogram of animal | | |
| | *kgm.* | *mgm.* | *mgm.* | | | | |
| 35 | 2.0 | 0.0000035 | 0.0000015 | $\frac{1}{100}$ | $\frac{1}{100}$ | 8 | Very faint, if any |
| 34 | 2.75 | 0.00003 | 0.00001 | $\frac{1}{20}$ | $\frac{1}{25}$ | 16 | Negative |
| 46 | 2.155 | 0.000001 | 0.00000045 | $\frac{1}{450}$ | $\frac{1}{550}$† | 15 | Negative; liberation of 0.000001 mgm. epinephrin per kilogram per minute (or $\frac{1}{850}$ of normal) could have been detected |
| 52 | 3.87 | 0.0000009 | 0.00000025 | $\frac{1}{700}$ | $\frac{1}{1000}$† | 20 | Negative |
| 31 | 2.63 | 0.00001 | 0.00004 | $\frac{1}{625}$ | $\frac{1}{650}$ | 105 | Negative |
| 32 | 4.14 | 0.00002 | 0.000005 | $\frac{1}{30}$ | $\frac{1}{50}$ | 105 | Positive but slight |
| 33 | 3.635 | 0.00002 | 0.0000055 | $\frac{1}{30}$ | $\frac{1}{45}$ | 106 | Positive |

* Since one of our objects in the previous investigation was to determine whether the whole secretion of epinephrin is dependent upon the integrity of the nerves, the results were expressed not only in fractions of a milligram per minute per animal, but also as fractions of a milligram per minute per kilogram of animal on the supposition that the animal still had two adrenals secreting at the same rate as the remaining gland. This showed when compared with the normal output of cats with both adrenals intact the extent to which the output of the remaining adrenal had been reduced by the operation. The residual liberation from the one adrenal expressed as a fraction of the normal liberation by one adrenal is obtained from the table by halving the denominators of the fractions in the fifth and sixth columns.

† There was no evidence that any epinephrin was being given off in these animals.

theless, in each animal the epinephrin output was determined at the end of the series of observations by the methods and under the experimental conditions previously employed for the normal cats. The results of the epinephrin estimations on the seven cats used for the blood sugar experiments after interference with the epinephrin output

are given in table 2. The adrenalin solution employed for the blood assay was always itself freshly assayed by the method of Folin, Cannon and Denis.

TABLE 2

| NUMBER OF CAT | WEIGHT | EPINEPHRIN OUTPUT | | FRACTION OF NORMAL LIBERATION | | DAYS AFTER OPERATION | EYE REACTIONS |
|---|---|---|---|---|---|---|---|
| | | Per minute | Per kilogram per minute | Per animal | Per kilogram of animal | | |
| | *kgm.* | *mgm.* | *mgm.* | | | | |
| 90 | 1.5 | 0.0000035 | 0.000002 | $\frac{1}{200}$ | $\frac{1}{120}$* | 65 | Negative |
| 108 | 2.2 | 0.000005 | 0.000002 | $\frac{1}{130}$ | $\frac{1}{120}$* | 35 | Negative. Could not have been 0.000005 mgm. per kg. per minute, or $\frac{1}{120}$ normal as determined by eye reactions |
| 109 | 3.22 | 0.00001 | 0.000003 | $\frac{1}{60}$ | $\frac{1}{80}$ | 39 | Negative. Could not have been $\frac{1}{60}$ of normal output per kg. per minute |
| 107 | 1.27 | 0.00013 | 0.00001 | $\frac{1}{50}$ | $\frac{1}{25}$ | 34 | Positive |
| 91 | 2.06 | 0.0001 | 0.00005 | $\frac{1}{6}$ | $\frac{1}{5}$ | 61 | Positive ($\frac{1}{4}$ of normal output per kg. per minute) |
| 92 | 1.93 | 0.00002 | 0.00001 | $\frac{1}{80}$ | $\frac{1}{60}$* | 61 | Negative |
| 121† | 1.7 | 0.000015 | 0.00001 | $\frac{1}{40}$ | $\frac{1}{25}$ | 24 | Negative. The output could not have been more than $\frac{1}{30}$ of normal for the whole animal, or $\frac{1}{20}$ of normal per kg. of animal |

* There was no evidence that any epinephrin was being given off.

† Left adrenal excised; nerves of right adrenal cut.

It will be seen that the results are precisely the same in these seven cats as in the seven reported in table 1. In two of them no epinephrin whatever was detected in the adrenal vein blood by the segment tests. No attempt was made to fix the minimum concentration of epinephrin which the segments could detect but it was shown that good reactions were still given with concentrations which with the observed blood flows through the adrenals would have corresponded to an output per kilogram of body weight per minute one hundred and twenty times less than the average for normal cats. There is no doubt that the quantity which could possibly have been present was still smaller. In cat 108 the eye tests gave as the possible maximum output the same fraction, $\frac{1}{120}$ of the normal, as the intestine tests. Again, it is extremely unlikely that had the reactions been a little more sensitive they would have detected epinephrin. It is much more probable that just as in the series shown in table 1, the fortunate coincidence

of specially sensitive test objects and blood specimens from animals in which the secretory nerves had been severed with unusual completeness would have enabled us to drive down the limit of the possible epinephrin output far beyond that actually obtained. The question, however, is of no consequence for our purpose. For an animal which cannot be liberating $\frac{1}{500}$ of the normal amount of epinephrin, owing to section of secretory nerve fibers, is certainly no more capable of responding to stimulation of any remaining fibers by an outburst, bringing the output far above the normal, than if it had been shown that the rate of liberation had been reduced to $\frac{1}{1000}$ or $\frac{1}{1500}$ of the normal. It must always be remembered that no evidence was obtained in these animals that any epinephrin was being given off. It is obvious that in connection with the problem whether an experimental hyperglycemia depends upon increased epinephrin secretion, experiments in which no epinephrin has been detected with sensitive test objects are more important than those in which a small residual liberation is still present. In a third cat of this series (cat 92) the result of the epinephrin assay was also negative. But here there were only the eye reactions to go by, not enough blood having been obtained for satisfactory segment tests. Still, the eye reactions were quite sensitive and showed that the output per kilogram per minute could not have been $\frac{1}{10}$ of the normal average.[1]

In one animal of the series (cat 91) a substantial fraction of the normal average output of epinephrin was found, $\frac{1}{3}$ or $\frac{1}{6}$ by the intestine segments and $\frac{1}{4}$ by the eye tests. There was no question that a marked diminution in the output per minute had been effected by the operation in this animal. For the concentration of epinephrin in the adrenal vein blood was far less than is ever seen in a normal cat under our experimental conditions, for the corresponding rates of adrenal blood flow. As the epinephrin assay was made sixty-one days after the nerve section, the possibility of some regeneration of the secretory fibers might be considered but we have no evidence as to this. If any regeneration had occurred in this time, the output of epinephrin determined at the end of the period would be greater than it was after section of the nerves. However, since this animal yielded precisely the same results as the others in the blood sugar observations, the question is of no significance for our present purpose.

To sum up, if tables 1 and 2 are compared it will be seen that the results of the operation, as practiced by us, upon the residual epinephrin secretion are of the same general character for the two series. In each group of seven cats two gave no evidence with either test of any epinephrin output whatever. In each group one cat showed a somewhat substantial residual liberation, although only a mere fraction of the

[1] Generally the eye reactions, as stated in a previous paper (3), will not detect such small outputs of epinephrin as the segment tests with shed blood. This depends, however, not only upon the threshold concentration and quantity of epinephrin which yield a just detectable reaction, but also upon the length of time during which it is feasible to continue the collection of the adrenal vein blood in the cava pocket, and this in turn depends upon the rate of blood flow through the gland.

normal. The remaining cats in each group were proved to be giving off a very small amount of epinephrin ($\frac{1}{40}$, $\frac{1}{50}$ down to $\frac{1}{200}$ of the normal), except cat 92 in the second group (table 2), which by the only tests applied, the eye reactions, yielded a negative result. If we reflect that to ensure the severing of the secretory innervation of the adrenal a far larger number of fibers which have nothing to do with the epinephrin secretion must be cut, it will readily be seen that the completeness with which the fibers in question are divided may vary in the different operations. Let this be as it may, the tables demonstrate conclusively that to all intents and purposes the epinephrin secretion by the adrenals may be considered as non-existent in cats after this operation and that any effect produced upon the blood sugar content by given conditions cannot be mediated through the nervous mechanism which normally governs the liberation of epinephrin from these glands.

One other remark may be made before passing from the consideration of these tables. It will be noticed that in table 2, the fraction of the normal output represented by the residual liberation expressed per kilogram of body weight is in general greater than the fraction expressed for the whole animal, while the opposite is the case in table 1. The reason for this, or at least the main reason, is a purely artificial one, namely that in table 2 the body weight of most of the cats is less than the body weight of the majority of those in table 1. This is partly a matter of accident but partly due to the fact that the cats in table 1, after the loss of weight which always occurs in the first weeks after the operation, remaining undisturbed by further interference, rapidly regained their original weight and in several instances became considerably heavier than before the operation. The animals in table 2 were used for the blood-sugar observations, samples of blood being repeatedly taken from them; they were subjected to periods of asphyxia or anesthesia, and naturally most of them lost some weight.

### THE NORMAL BLOOD-SUGAR CONTENT IN THE CAT

It has already been stated that in determining whether an increase in the sugar content of the blood was caused by the conditions investigated, comparison was not made with an average "normal" content deduced from observations made at other times on the same or on other animals, but successive samples collected at the time of each experiment were compared. In testing out the technique, however, a number of normal sugar estimations were first made. Some of these are given in table 3, with the duplicate estimations; in the rest of the paper only the average of the duplicate observations is given. For convenience, estimations on two dogs are included in the table.

TABLE 3

| NUMBER OF ANIMAL | PERCENTAGE OF DEXTROSE IN BLOOD | | | REMARKS |
|---|---|---|---|---|
| | Duplicates | | Average | |
| 1 | 0.106 | 0.115 | 0.11 | Excited during collection |
| 2 | 0.081 | 0.083 | 0.082 | Considerably excited |
| 3 | 0.108 | 0.108 | 0.108 | Considerably excited (lost 10 cc. blood) |
| 4 | 0.117 | 0.116 | 0.116 | Struggled and cried |
| 5 | 0.107 | 0.111 | 0.109 | Very quiet |
| 7 | 0.103 | 0.103 | 0.103 | Excited |
| 11 | 0.081 | 0.081 | 0.081 | Quiet |
| 19* | 0.094 | 0.097 | 0.096 | Much excited; blood dark |
| 15† | 0.113 | 0.114 | 0.114 | Very quiet |
| 16† | 0.105 | 0.104 | 0.104 | Apprehensive |

* This cat was given no liver for two days before the blood samples were obtained, and no food at all for twenty-four hours, two days before the blood experiment. All the others were on the liver diet with milk daily, but no rice.

† Dogs: no. 15, a large female hound; no. 16, a small female fox terrier

Scott (4) has published numerous blood-sugar estimations on cats. He worked with large quantities of blood obtained by decapitation, precipitating the proteins by a special method and estimating the sugar by a method described by Munsen and Walker (5). Our "normal" results for cats agree fairly well with those given by him but are on the whole somewhat higher. We have not employed dogs for the adrenal operations because the nerve paths whose section abolishes or greatly lessens the epinephrin output are better known in the cat than in the dog.

*Experiments on cats in which the epinephrin output was interfered with*

*Cat 90.* Condensed protocol. Weight, 1.82 kgm. Right adrenal excised and nerves of left adrenal cut on May 9.

| | BODY-WEIGHT | | PERCENTAGE OF BLOOD-SUGAR |
|---|---|---|---|
| | *kgm.* | | |
| May 22 | 1.625 | Moderate asphyxia, 10 minutes | 0.17 |
| June 11 | 1.605 | "Normal" blood. Restless | 0.09 |
| June 30 | 1.495 | After being tied on board 3 minutes | 0.129 |
| | | After being tied on board 15 minutes | 0.128 |
| June 28 | 1.665 | Excised left superior cervical ganglion | |

| | WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|
| | | Preliminary specimen | Ether | Asphyxia |
| | *kgm.* | | | |
| June 21..... | 1.515 | 0.20* | 0.233 | |
| June 27..... | 1.67 | 0.087 | 0.151 | |
| July 2..... | 1.78 | 0.07 | | 0.168 |
| July 7..... | 1.635 | 0.089 | | 0.164 |

* During collection of this specimen the blood flowed very slowly and was very dark.

*July 13.* Weight, 1.5 kgm.
10.00 a.m. 3 grams urethane by stomach tube.
10.30 a.m. Prepared cava pocket, tying all arteries, i.e., renal, coeliac and mesenteric arteries and abdominal aorta.
11.20 a.m. Pocket experiment, 2 minutes occlusion; no eye reactions.
11.30 a.m. Pocket experiment, 4 minutes occlusion: no eye reactions.
11.35 a.m. Pocket experiment, 6 minutes occlusion: no eye reactions.
11.38 a.m. 0.5 cc., 1: 1,000,000 adrenalin injected; very slight pupil, no nictitating reaction.
11.45 a.m. Repeated last observation with same result.
Then collected the following specimens of adrenal blood:
First specimen, 1.5 grams in 1½ minutes, blood flow 1 gram per minute.
Second specimen, 4.5 grams in 5 minutes, blood flow 0.9 gram per minute.
Third specimen, 5.8 grams in 7 minutes, blood flow 0.83 gram per minute.
Right adrenal weighed 0.206 gram, and contained 0.18 mgm. epinephrin.
Left adrenal weighed 0.186 gram, and contained 0.17 mgm. epinephrin.

Some of the tracings illustrating the epinephrin assay are reproduced in figures 1 to 3. Figure 1 shows that the second adrenal specimen diluted with three volumes of Ringer's solution caused no inhibition of the intestine (observation 21), and that the undiluted blood could not have contained 1: 150,000,000 epinephrin since indifferent blood containing this amount caused a distinct inhibition (observation 23). That the limit must have been decidedly lower than 1: 150,000,000 is shown by the fact that in reducing the concentration from 1: 90,000,000 (fig. 2, observation 13), to 1: 150,000,000 (fig. 1) only a moderate reduction takes place in the inhibitory effect, whereas the difference between the effect of 1: 30,000,000 (fig. 2, observation 11) and 1: 90,000,000 is very great. In figure 3, it is proved that indifferent blood containing a concentration of 1: 200,000,000 adrenalin (observation 41) causes a much greater increase of tone of a uterus segment than the second adrenal blood specimen (observation 37), or than the third specimen (observation 39). The comparison of observations 36 and 42

shows that this uterus segment could detect a concentration of 1:300,000,000. We can conclude that the second adrenal specimen does not contain 1:200,000,000, probably not 1:300,000,000. It is certainly quite safe to assume that it could not have contained 1:250,000,000. Therefore, the output of epinephrin could not have been 0.0000035 mgm. per minute, i.e., 0.000002 mgm. per kilo of body weight per minute, or $\frac{1}{120}$ of the normal average output per kilogram, as determined by rabbit segments on shed blood. There was no evidence that any epinephrin was being given off.

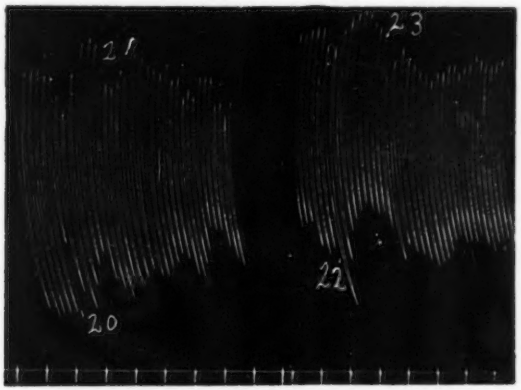


Fig. 1. Intestine tracings. Bloods from cat 90. At *20* Ringer was replaced by indifferent (arterial) blood, and this at *21* by the second adrenal blood specimen. Both bloods were diluted with three volumes of Ringer's solution. At *22* Ringer's solution was replaced by indifferent blood diluted with three volumes Ringer, and this at *23* by the indifferent blood made up with adrenalin to a concentration of 1:150,000,000, the mixture being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

*Cat 108.* Condensed protocol. Female. Weight, 2.75 kgm. Right adrenal excised and nerves of left adrenal cut July 6.

| | WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|
| | | Preliminary specimen | Fright | Asphyxia |
| | *kgm.* | | | |
| July 17 | 2.4 | 0.074 | | 0.129 |
| July 26 | 2.475 | 0.105 | 0.093 | 0.165 |
| August 6 | | 0.078 | 0.09 | |

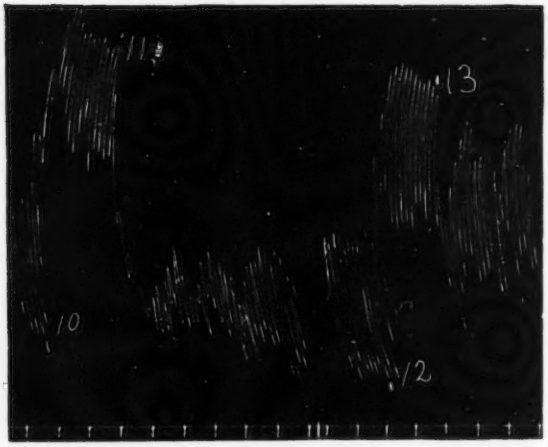


Fig. 2. Intestine tracings. Bloods from cat 90. At *10* and *12* Ringer's solution was replaced by indifferent blood diluted with three volumes Ringer, and this at *11* and *13* by the indifferent blood made up with adrenalin to a concentration of 1: 30,000,000 and 1: 90,000,000, respectively, the adrenalin bloods being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

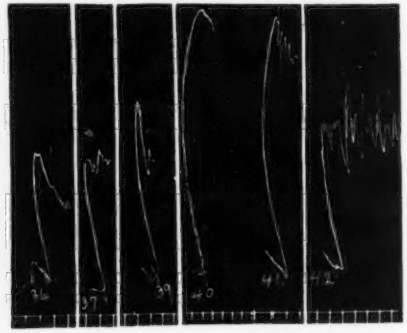


Fig. 3. Uterus tracings. Bloods from cat 90. At *36* Ringer was replaced by indifferent (arterial) blood; at *37* by the second adrenal specimen; at *39* by the third adrenal specimen; at *40* by the indifferent blood to which adrenalin had been added to make up a concentration of 1: 100,000,000; at *41* by the indifferent blood to which adrenalin had been added to make up 1: 200,000,000; at *42* by the indifferent blood to which adrenalin had been added to make up 1: 300,000,000. All the bloods were undiluted. (Reduced to one-half.)

August 2. Excised left superior cervical ganglion.
August 10. Weight, 2.2 kgm. Made cava pocket under urethane (4 grams), tying all arteries.
1.10 p.m. Pocket experiment 4 minutes: no eye reactions.
1.16 p.m. Pocket experiment 6 minutes: no eye reactions.
1.25 p.m. 0.5 cc. adrenalin, 1: 1,200,000 injected: very good eye reactions in 7.2 seconds.
1.28 p.m. 0.5 cc. adrenalin, 1: 2,300,000 injected: good eye reactions in 8 seconds.
1.34 p.m. 0.5 cc. adrenalin, 1: 4,500,000 injected: slight eye reactions in 10.2 seconds.
1.38 p.m. 0.5 cc. adrenalin, 1: 7,000,000 injected: small retraction of nictitating in 11.4 seconds, no pupil reactions.
Now collected the following specimens of adrenal blood:
First specimen, 3.2 grams in 1 minute, blood flow 3.2 grams per minute.
Second specimen, 7.8 grams in 3.5 minutes, blood flow 2.2 grams per minute.
Third specimen, 3.0 grams in 3 minutes, blood flow 1.0 gram per minute.
Then obtained blood from abdominal aorta.
Right adrenal weighed 0.176 gram, and contains 0.23 mgm. epinephrin.
Left adrenal weighed 0.278 gram, and contained 0.26 mgm. epinephrin.

Some of the tracings of the epinephrin assay are reproduced in figures 4 to 6. In figure 4 it is shown that the second adrenal specimen (observation 4) gave no inhibition of the intestine when diluted with an equal volume of Ringer's solution. Another observation, not reproduced, proved that even when undiluted it caused no inhibition, which was also true of the third adrenal specimen (observation 14). Observation 16 (fig. 5) indicates that even the third specimen could not have contained nearly 1: 200,000,000 epinephrin. Comparison of observations 10 and 16 suggests that the limit of sensitiveness of the intestine segment had not been nearly reached with a concentration of 1: 200,000,000, since the inhibitory effect at 16 is far from insignificant as compared with that at 10. In figure 6 it is demonstrated by uterus tests that the second adrenal specimen (observation 21) contained less epinephrin than 1: 230,000,000 (observation 20). Indeed, the effect of this sample on the uterus was not greater than that of the indifferent blood (observation 19). Taking the rate of blood flow through the adrenals during collection of the third specimen as 1 cc. per minute, it follows that the output of epinephrin per minute could not have been nearly as much as 0.000005 mgm. per minute for the animal, or 0.000002 mgm. per kilogram of body weight per minute, i.e., less than $\frac{1}{1\frac{1}{2}0}$ of the average output of normal cats, as determined on rabbit intestine and uterus segments. If any epinephrin at all was being given off, of which there was no evidence, the amount must have been decidedly

less than this. The eye reactions, which in this animal were very sensitive, also gave a completely negative result, even for a six-minute occlusion of the pocket. From these reactions it can also be calculated that the possible output could not have been $\frac{1}{120}$ of the normal as determined by the eye tests.

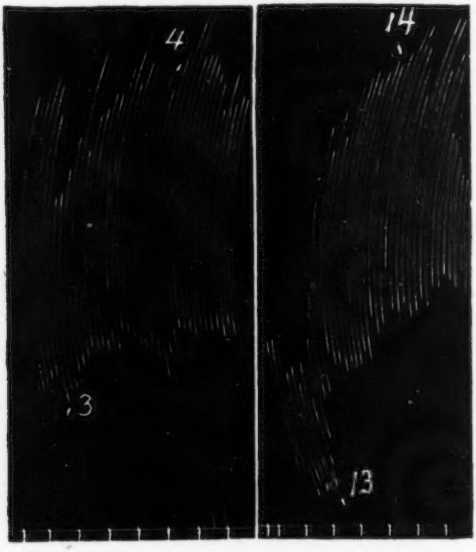


Fig. 4. Intestine tracings. Bloods from cat 108. At *3* Ringer's solution was replaced by indifferent (arterial) blood, and this at *4* by the second adrenal blood specimen. Both bloods were diluted with one volume Ringer before application to the segment. At *13* Ringer was replaced by the indifferent blood undiluted, and this at *14* by the third adrenal blood sample also undiluted. After observation 4 was completed the drum and writing point were lowered. (Reduced to two-thirds.)

*Cat 92.* Condensed protocol. Female. Weight, 2.525 kgm. Right adrenal excised and nerves of left cut May 9.

May 21. Weight 2.075 kgm. Blood sugar after light ether anesthesia, 0.154 per cent.

May 29. Excised left superior cervical ganglion.

June 20. Weight, 2.285 kgm., tied on board 3 minutes. Blood sugar, 0.098 per cent. After being on board 28 minutes, blood sugar, 0.108 per cent.

| | WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|
| | | Preliminary specimen | Asphyxia | Light ether |
| | *kgm.* | | | |
| June 18..... | 2.395 | 0.096 | 0.104 | |
| June 27..... | 2.06 | 0 093 | 0.151 | |
| July 2..... | 1.96 | 0.064 | 0.092 | |
| July 7..... | 2.0 | 0.142* | | 0.176 |

* Very slow collection and serum separated in the pipette.

July 9. Weight, 1.93 kgm., cava pocket formed under urethane (4 grams) with all arteries tied.

12.50 p.m. Pocket experiment, 3 minutes: no eye reactions.

12.55 p.m. Pocket experiment, 5 minutes: no eye reactions.

1.05 p.m. 0.5 cc. adrenalin, 1: 1,000,000 injected; very good eye reactions in 10.2 seconds.

1.08 p.m. 0.25 cc. adrenalin, 1: 1,000,000 injected: good eye reactions in 10.4 seconds.

1.10 p.m. 0.5 cc. adrenalin, 1: 3,000,000 injected: small pupil; no nictitating reaction, 11.6 seconds.

1.18 p.m. 0.5 cc. adrenalin, 1: 4,000,000 injected: small pupil and nictitating reaction, 10.8 seconds.

1.20 p.m. 0.5 cc. adrenalin, 1: 5,000,000 injected: small pupil and nictitating reaction, 11.4 seconds.

A small specimen of adrenal blood was obtained, but not sufficient for assay, as the cat died shortly after collection was begun.

Left adrenal weighed 0.250 gram, and contained 0.19 mgm. epinephrin.

Right adrenal was crushed in removal, no assay.

In this animal the eye tests showed that a reaction could be gotten with 0.0001 mgm. epinephrin, whereas the adrenal vein blood collected for five minutes and then released produced no reaction whatever. Accordingly, the output of epinephrin could not have been as great as 0.00002 mgm. per minute, i.e., 0.00001 mgm. per kilogram of body weight per minute. This is not $\frac{1}{6}\frac{1}{0}$ of the average output in normal cats, as determined by eye reactions. There was no evidence that any epinephrin was being given off.

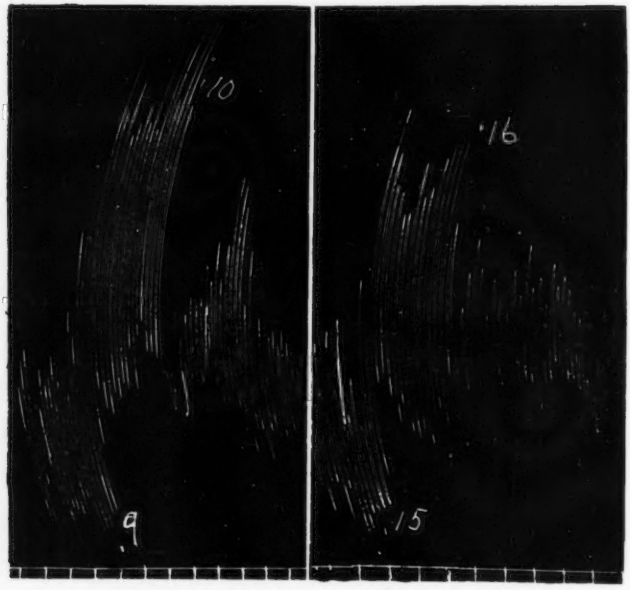


Fig. 5. Intestine tracings. Bloods from cat 108. At *9* and *15* Ringer's solution was replaced by the indifferent blood (undiluted), and this at *10* and *16* by the indifferent blood made up with adrenalin to a concentration of 1: 115-000,000 and 1: 200,000,000 respectively. (Reduced to two-thirds.)

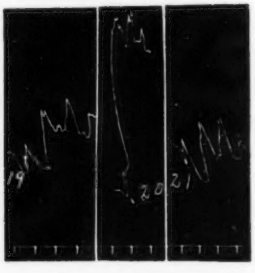


Fig. 6. Uterus tracings. Bloods from cat 108. At *19* Ringer's solution was replaced by the indifferent blood; at *20* by the indifferent blood made up with adrenalin to a concentration of 1: 230,000,000; at *21* by the second adrenal specimen. All the bloods were undiluted. (Reduced to one-half.)

*Cat 109.* Condensed protocol. Male. Weight, 4.65 kgm. Right adrenal excised and nerves of left cut, July 6.

| | WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|
| | | Preliminary specimen | Fright | Asphyxia |
| | *kgm.* | | | |
| July 17..... | 3.64 | 0.07 | 0.075 | 0.14 |
| July 26..... | 3.245 | 0.086 | 0.076 | 0.183 |

August 2. Excised left superior cervical ganglion.

August 14. Weight, 3.22 kgm., cava pocket formed under urethane (5 grams), all arteries tied.

12.26 p.m. Pocket experiment, 3 minutes: no eye reactions.

12.30 p.m. Pocket experiment, 6 minutes: no eye reactions.

0.5 cc. adrenalin, 1:2,300,000 injected: slight nictitating reaction in 11.2 seconds.

0.5 cc. adrenalin, 1:1,150,000 injected: good pupil and nictitating in 8.2 seconds.

Now collected the following adrenal blood specimens:

First specimen, 2.1 grams in 1 minute, blood flow 2.1 grams per minute.[2]

Second specimen, 6.9 grams in 6 minutes, blood flow 1.15 grams per minute.

Third specimen, 3.4 grams in 5 minutes, blood flow 0.7 gram per minute.

Obtained blood from abdominal aorta.

Right adrenal weighed 0.365 gram and contained 0.38 mgm. epinephrin.

Left adrenal weighed 0.331 gram and contained 0.34 mgm. epinephrin.

Some of the tracings of the epinephrin assay are given in figures 7 to 9. The second adrenal specimen diluted with three volumes of Ringer's solution (fig. 7, observation 2) gave no inhibition of the intestine, while indifferent blood containing 1:35,000,000 adrenalin similarly diluted gave a good inhibition. In figures 8 and 9 it is proved that the undiluted second adrenal blood specimen, while causing distinct inhibition of the intestine, could not have contained 1:85,000,000 epinephrin though somewhat more than 1:115,000,000. Taking the concentration as the average of these two observations, i.e., 1:100,000,000, we get 0.00001 mgm. as the output per minute or 0.000003 mgm. per kilogram of body weight per minute. This is only $\frac{1}{80}$ of the average output in normal cats, as estimated by rabbit segments in drawn blood.

[2] The first small sample is collected apart, in order to get rid of any epinephrin which may have been liberated by manipulation when the upper end of the pocket is being clipped off. The much greater apparent rate of blood flow sometimes seen in the collection of this sample is partly due to the inclusion of some blood already in the pocket when the clamp is applied.

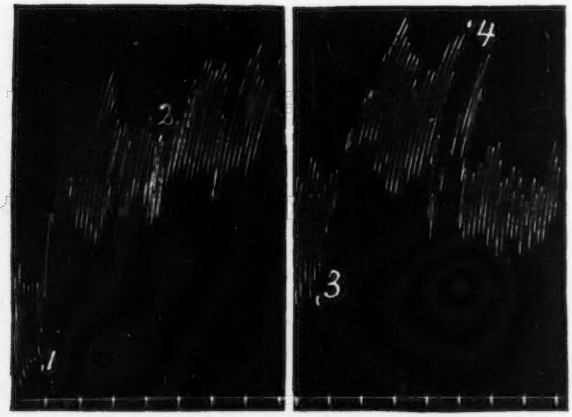


Fig. 7. Intestine tracings. Bloods from cat 109. At *1* Ringer's solution was replaced by indifferent blood and this at *2* by the second adrenal blood specimen. Both bloods were diluted with three volumes Ringer. At *3* Ringer's was replaced by the indifferent blood (diluted with three volumes Ringer), and this at *4* by the indifferent blood made up with adrenalin to a concentration of 1: 35,000,000, the mixture being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

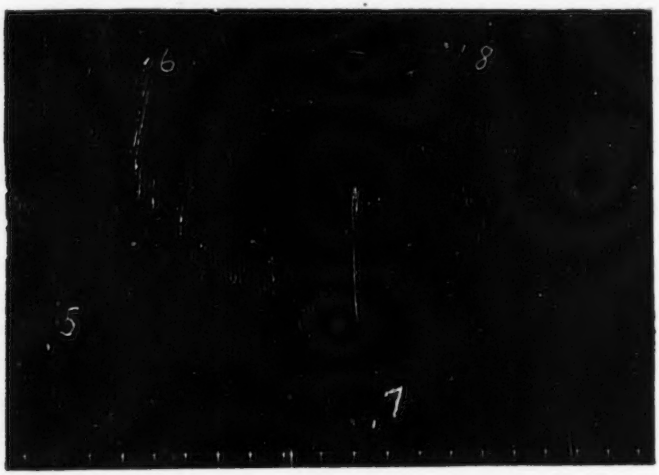


Fig. 8. Intestine tracings. Bloods from cat 109. At *5* Ringer's solution was replaced by the indifferent blood, and this at *6* by the second adrenal blood sample. At *7* Ringer was replaced by the indifferent blood, and this at *8* by the indifferent blood made up with adrenalin to a concentration of 1: 115,000,000. All the bloods were undiluted. (Reduced to two-thirds.)

The eye reactions were negative even for a six-minute collection of the adrenal vein blood although a reaction was obtained when as little as 0.0002 mgm. adrenalin was injected. It can be calculated from these data that the output per kilogram of body weight per minute could not have been $\frac{1}{6}\frac{1}{5}$ of the average output in normal cats, as determined by the eye reactions.

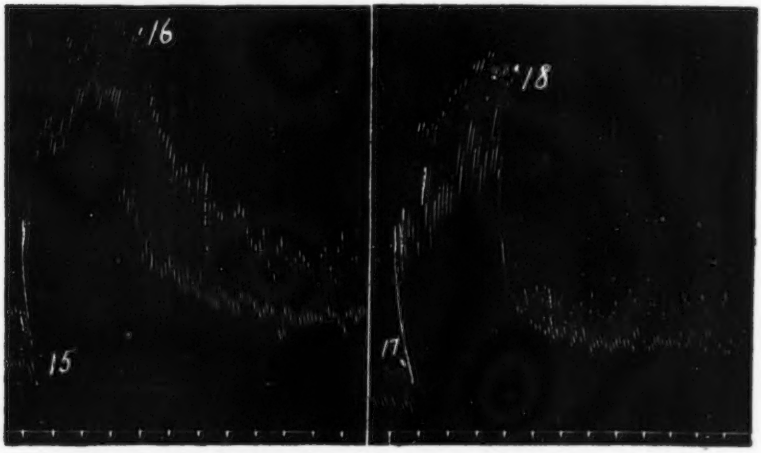


Fig. 9. Intestine tracings. Bloods from cat 109. At *15* Ringer's solution was replaced by the indifferent blood, and this at *16* by the second adrenal blood sample. At *17* Ringer was replaced by the indifferent blood, and this at *18* by the indifferent blood to which adrenalin had been added to make up 1: 85,000,000. All the bloods were undiluted. The weight was less than in figure 8. (Reduced to two-thirds.)

*Cat 107.* Condensed protocol. Female. Weight 1.82 kgm. Right adrenal excised and nerves of left cut, July 6.

August 2. Excised left superior cervical ganglion.

| | WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|
| | | Preliminary specimen | Fright | Asphyxia |
| | *kgm.* | | | |
| July 17..... | 1.5 | 0.075 | | 0.247 |
| August 6..... | 1.275 | 0.086 | 0.078 | 0.160 |

August 9. Weight 1.27 kgm. Under urethane (3 grams) cava pocket formed with all arteries tied. Positive pupil reactions were obtained on collecting adrenal vein blood in the cava pocket and then releasing it, but there was no movement of the nictitating membrane. The following samples of adrenal blood were collected.

First sample, 1.1 grams in 1 minute, blood flow 1.1 grams per minute.

Second sample, 3.8 grams in 5 minutes, blood flow 0.8 gram per minute.

Third sample, 3.3 grams in 7 minutes, blood flow 0.47 gram per minute.

Indifferent blood was obtained from the abdominal aorta.

Right adrenal weighed 0.168 gram and contained 0.23 mgm. epinephrin.

Left adrenal weighed 0.152 gram and contained somewhat more than 0.12 mgm. epinephrin.

Figure 10, in which a few of the tracings of the epinephrin assay are reproduced, shows that even the third adrenal specimen, in spite of the relatively small flow of blood during its collection, had scarcely 1:35,000,000 epinephrin and much less than 1:17,000,000. The epinephrin output was therefore less than 0.000015 mgm., i.e., about 0.00001 mgm. per kilogram of body weight per minute. This is not more than $\frac{1}{25}$ of the average output of normal cats per kilogram. It is only because of the slow blood flow that the concentration is even as great as 1:35,000,000.

*Cat 91.* Condensed protocol. Female. Weight, 2.23 kgm. Right adrenal excised and nerves of left cut, May 9.

May 29. Weight 2.00 kgm., dextrose in blood, 0.105 per cent.

May 29. Excised left superior cervical ganglion.

June 20. Weight, 1.95 kgm. Tied on board 3 minutes, blood-sugar, 0.108 per cent. Still tied on board 12 minutes, blood-sugar, 0.108 per cent.

| | WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|
| | | Preliminary specimen | Light ether | Asphyxia |
| | *kgm.* | | | |
| June 21..... | 2.0 | 0.092 | 0.98 | |
| July 2..... | 2.145 | 0.077 | | 0.10 |
| July 7..... | 2.05 | 0.116 | | 0.321 |

July 9. Weight 2.065 kgm. Under urethane (4 grams) cava pocket formed with all arteries tied.

4.23 p.m. Pocket experiment, 2 minutes: slight pupil reaction in 9 seconds.

4.25 p.m. Pocket experiment, 2 minutes: slight pupil reaction in 11.6 seconds.

4.28 p.m. Pocket experiment, 4 minutes: small pupil and nictitating reaction in 10 seconds.

4.35 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected: good pupil, slight nictitating in 9.2 seconds.

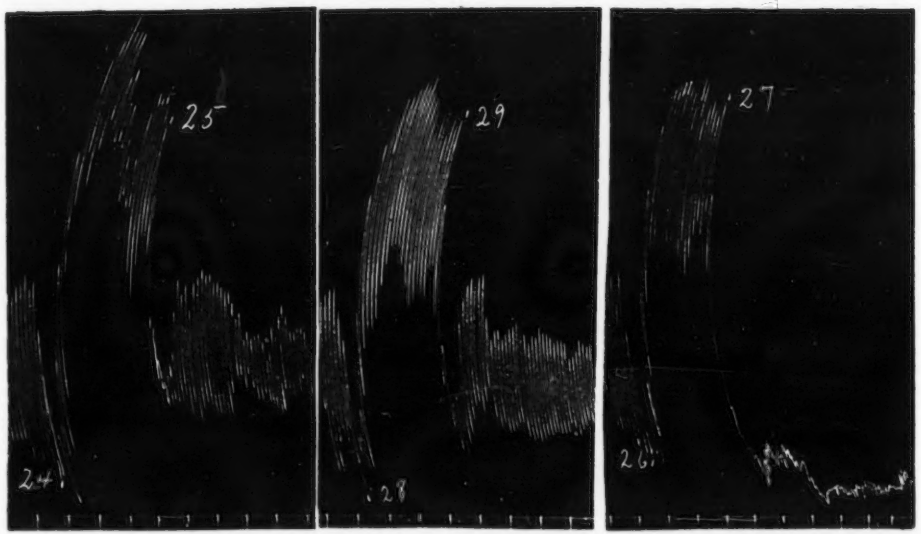


Fig. 10. Intestine tracings. Bloods from cat 107. At *24* Ringer was replaced by indifferent (arterial) blood, and this at *25* by the third adrenal blood sample. Both bloods were diluted with one volume of Ringer. At *26* Ringer was replaced by the indifferent blood diluted with an equal volume of Ringer and this at *27* by the indifferent blood made up with adrenalin to 1: 17,000,000, and then diluted with an equal volume of Ringer. At *28* Ringer was replaced by the indifferent blood diluted with an equal volume of Ringer, and this at *29* by the indifferent blood made up with adrenalin to a concentration of 1: 35,000,000 and then diluted with an equal volume of Ringer. (Reduced to two-thirds.)

4.40 p.m. 0.5 cc. adrenalin, 1: 2,000,000 injected: small pupil in 12.2 seconds (like observation at 4.28).

4.45 p.m. Pocket experiment, 4 minutes; pupil reaction in 8.2 seconds, greater than at 4.40, more like that at 4.35.

4.48 p.m. 0.5 cc. adrenalin, 1: 1,000,000: slight pupil reaction in 12.4 seconds.

4.57 p.m. Pocket experiment, 2 minutes: small pupil reaction in 8.6 seconds.

5.00 p.m. 0.5 cc. adrenalin, 1: 1,000,000 injected; good pupil reaction in 10.8 seconds (larger than in observation at 4.57).

5.07 p.m. Pocket experiment, 3 minutes; pupil reaction nearly the same as that in observations at 5.00.

A number of additional observations on the eye reactions with adrenalin injection, and adrenal vein blood released from the pocket were made. Then the following specimens of adrenal blood were drawn through a cannula in the cava and tested on rabbit intestine and uterus segments.

First specimen, 1.1 grams in 2 minutes, blood flow, 0.55 gram per minute.

Second specimen, 2.5 grams in 15 minutes, blood flow 0.17 gram per minute.

Third specimen, 1.8 grams in 23 minutes, blood flow 0.08 gram per minute.

The blood flowed very slowly.

Right adrenal weighed 0.232 gram and contained 0.26 mgm. epinephrin.

Left adrenal weighed 0.265 gram and contained somewhat more than 0.16 mgm. epinephrin.

In figures 11 and 12 are reproduced some of the tracings of the epinephrin assay. It gave much the greatest concentrations of epinephrin in the adrenal vein seen in the series of cats whose adrenal innervation had been interfered with. The second adrenal specimen had a concentration of epinephrin about equal to 1: 1,500,000 (fig. 11). It was distinctly greater than 1: 2,000,000. In the third specimen the concentration was greater than 1: 800,000, and less than 1: 500,000 (fig. 12). The blood had an unusually high proportion of plasma, not far from 90 per cent, and the flow during collection was very small. For both reasons the concentration is high. We have stated elsewhere (3), (6) that a concentration of more than 1: 1,000,000 is unusual in cats' adrenal vein blood, but the proportion in serum can be considerably higher since the erythrocytes contain practically no epinephrin. The output of epinephrin calculated for the second adrenal specimen was 0.0001 mgm. per minute for the animal and 0.00005 mgm. per kilogram per minute, or about $\frac{1}{5}$ of the normal output in the cat as determined on drawn blood by rabbits' intestine segments. Taking the concentration in the third specimen at 1: 650,000, we get 0.00012 mgm. per minute, or 0.00006 mgm. per kilogram of body weight per minute.

The eye reactions gave about 0.00015 mgm. per minute, or 0.00007 mgm. per kilogram of body weight per minute as the output, i.e., about $\frac{1}{3}$ or $\frac{1}{10}$ of the normal as determined by the eye tests.

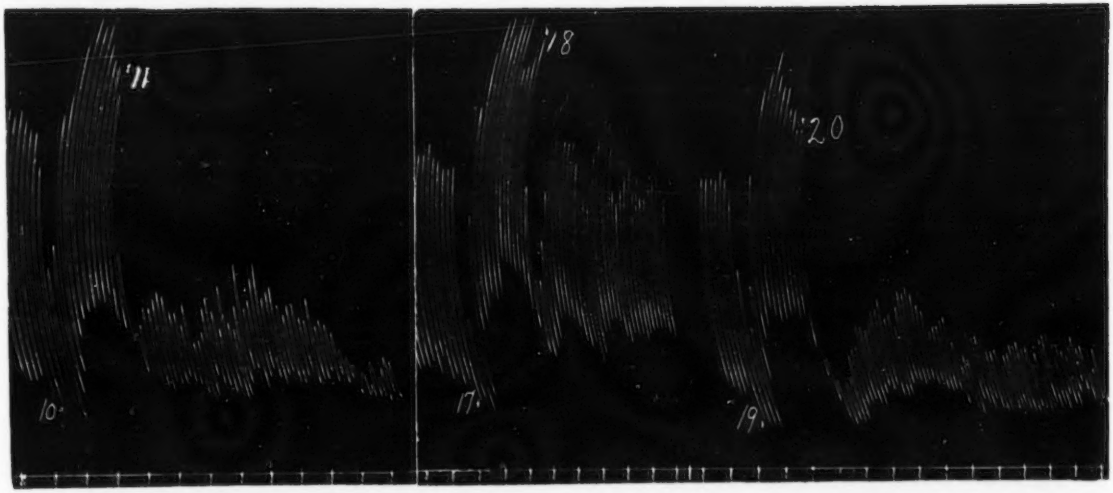


Fig. 11. Intestine tracings. Bloods from cat 91. At *10* and *17* Ringer's solution was replaced by indifferent (arterial) blood, diluted with five volumes Ringer, and this at *11* and *18* by the indifferent blood made up with adrenalin to concentrations of 1: 1,500,000 and 1: 2,000,000 respectively, the adrenalin bloods being then diluted with five volumes Ringer before application to the segment. At *19* Ringer's solution was replaced by the indifferent blood and this at *20* by the second adrenal blood specimen, both bloods being diluted with five volumes Ringer. (Reduced to two-thirds.)

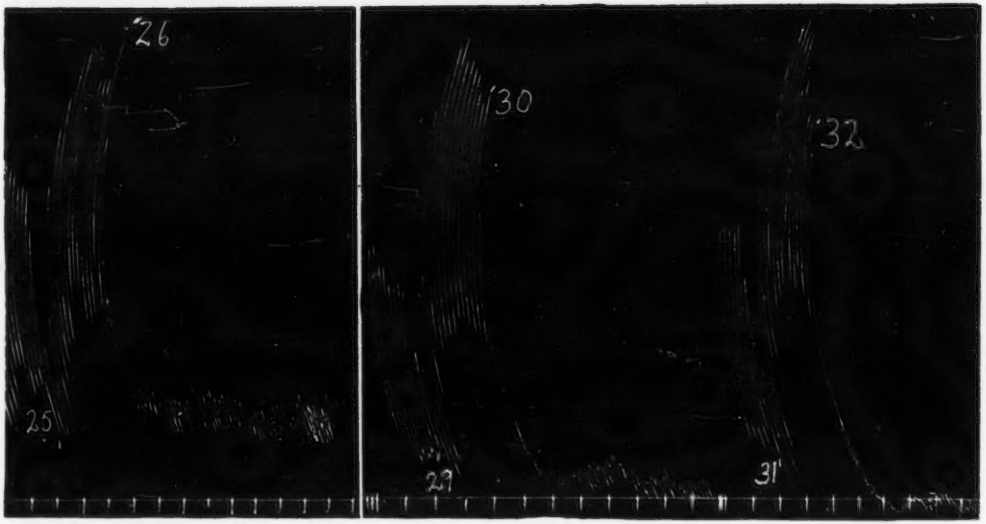


Fig. 12. Intestine tracings. Bloods from cat 91. At *25* and *31* Ringer's solution was replaced by the indifferent blood diluted with five volumes Ringer, and this at *26* and *32* by the indifferent blood made up with adrenalin to concentrations of 1: 800,000 and 1: 500,000 respectively, the adrenalin bloods being diluted with five volumes Ringer before application to the segment. At *29* Ringer's solution was replaced by the indifferent blood and this at *30* by the third adrenal blood sample, both bloods being diluted with five volumes Ringer. In observation *32* the writing point went below the drum and stayed down for a time. (Reduced to two-thirds.)

It is commonly stated that in the rabbit the left splanchnic is much more important than the right for the production of some of the experimental hyperglycemias. Although there is no reason to believe that such a difference exists in the cat, the left adrenal was removed in one of the animals (cat 121) and the nerves of the right adrenal, including of course the right splanchnic, cut. The results differed in no way from those obtained on the other cats.

*Cat 121.* Condensed protocol. Female. Weight, 1.975 kgm. Left adrenal excised and nerves of right cut, July 31.

August 13. Weight, 1.71 kgm. Preliminary blood specimen, 0.111 per cent dextrose. Asphyxia, 0.163 per cent dextrose.

August 16. Excised left superior cervical ganglion.

August 24. Weight, 1.70 kgm. Under urethane (3 grams) cava pocket formed, with all arteries tied.

11.35 a.m. Pocket experiment, 3½ minutes: no eye reactions.

11.40 a.m. Pocket experiment, 6 minutes: no eye reactions.

11.50 a.m. 0.5 cc. adrenalin, 1: 1,150,000: excellent eye reactions in 11.8 seconds.
0.5 cc. adrenalin, 1: 2,300,000: good eye reactions in 17.4 seconds.

Now collected the following specimens of adrenal vein blood:

First specimen, 0.8 gram in 1 minute, blood flow 0.8 gram per minute.

Second specimen, 3.6 grams in 6½ minutes, blood flow 0.5 gram per minute.

Third specimen, 5.2 grams in 11 minutes, blood flow 0.47 gram per minute.

Fourth specimen, 2.0 grams in 6 minutes, blood flow 0.33 gram per minute.

During collection of the fourth specimen, a clot had to be squeezed out of the pocket.

Right adrenal weighed 0.242 gram and contained 0.26 mgm. epinephrin.

Left adrenal weighed 0.152 gram and contained 0.16 mgm. epinephrin.

It was shown on the rabbit intestine and uterus segments that the third adrenal blood specimen contained about 1: 30,000,000 epinephrin. The rate of blood flow was approximately 0.45 cc. per minute. Accordingly the output of epinephrin per minute was about 0.000015 mgm. per minute for the animal or 0.00001 mgm. per kilogram of body weight per minute, i.e., $\frac{1}{2}\frac{1}{5}$ of the average normal output, as determined on drawn blood with rabbits' segments. The eye reactions were negative even for a six-minute collection although 0.0002 mgm. of adrenalin gave a reaction which was more than minimal. The output of epinephrin was therefore not above 0.000035 mgm. per minute for the animal (about $\frac{1}{4}\frac{1}{5}$ of the normal average), or 0.00002 mgm. per kilogram of body weight per minute, (about $\frac{1}{3}\frac{1}{0}$ of the normal average), as determined by eye reactions.

As regards the blood-sugar percentages, it will be seen from the protocols that asphyxia and etherization caused distinct hyperglycemia in the cats whose epinephrin output had been abolished, within the

limits of detection by the methods employed as well as in those in which a small residual output was still present. If the results on these cats are compared with those obtained in the same way on normal cats (tables 4 and 5) it will be seen that no essential difference can be made out. The precise amount of the hyperglycemia has no particular significance for it is well known that this is influenced greatly by the nutritive condition of the animals, particularly by the glycogen content of the liver. The same is true of the occasional failure to obtain the expected hyperglycemia, which is seen in the normal animals as well as in those subjected to the adrenal operation. It is conceivable that in the operated cats the loss of one major splanchnic, in addition to other sympathetic fibers, possibly going to the liver, if these nerves are at all concerned in the hyperglycemias studied, may have prevented the percentage of dextrose in the blood from rising as high after asphyxia or etherization as in the normal cats, although it is not cer-

TABLE 4

| NUMBER OF CAT | DATE | BODY WEIGHT | PERCENTAGE OF BLOOD-SUGAR | | |
|---|---|---|---|---|---|
| | | | Preliminary specimen | Fright | Asphyxia |
| | | *kgm.* | | | |
| 111 | July 19* | 2.4 | | | |
| | July 30 | 2.285 | 0.082 | 0.098 | 0.134 |
| | August 13 | 2.11 | 0.108 | | 0.225 |
| 112 | July 19* | 2.24 | | | |
| | July 30 | 2.015 | 0.097 | 0.105 | 0.282 |

* This was the date of the operation.

tain from the results that there is any decided deficiency in this respect in the former group.

In table 4 are given some observations on two cats in which a dummy operation was performed, as already mentioned, to imitate as far as possible the nutritive consequences of the adrenal operation, except that in these cats the adrenals were intact.

In table 5 are displayed the results of the blood-sugar estimations on a series of normal cats subjected to the same procedures as the cats which had undergone the adrenal operations.

From these results it seems impossible to draw any other conclusion than that the hyperglycemia associated with asphyxia and with etherization is not produced through the intervention of epinephrin liberated from the adrenal glands, or at least that the liberation of epi-

TABLE 5

| NUMBER OF CAT | DATE | BODY WEIGHT | PERCENTAGE OF DEXTROSE IN BLOOD | | | |
|---|---|---|---|---|---|---|
| | | | Normal or preliminary sample | Fright | Ether | Asphyxia |
| | | *kgm.* | | | | |
| 5 | May 18 | 2.36 | 0.109 | | | |
| | June 12 | 2.065 | | | | 0.163 |
| | June 27 | 2.43 | 0.096* | | | 0.321 |
| 6 | May 19 | 3.065 | | | 0.332† | |
| | June 16 | 2.124‡ | | | | 0.422-A§ |
| | | | | | | 0.510-B |
| 8 | June 1 | 2.585 | | 0.11‖ | | |
| 9 | May 22 | 2.815 | | | | 0.226 |
| 10 | May 22 | 2.53 | | | 0.277 | |
| 17 | June 27 | 2.68 | 0.089■ | | 0.157 | |
| | July 18 | 2.50 | 0.100 | | | 0.257 |
| 21 | June 18 | 2.97 | 0.131 | 0.171‖ | | |
| | July 3 | 2.89 | 0.078 | | | 0.079 |
| | July 18 | 2.865 | 0.122 | | | 0.201 |
| 22 | June 18 | 2.775 | 0.109 | | | 0.128 |
| | June 20 | 2.70 | 0.115-A• | | 0.161 | |
| | | | 0.101-B | | | |
| | July 3 | 2.525 | 0.080 | | | 0.096 |
| 23 | July 18 | 1.910 | 0.081 | 0.157‖ | | 0.214 |
| 24 | July 24 | 2.140 | 0.086 | 0.104 | | 0.329 |
| 26 | July 3 | 2.630 | 0.095 | | | 0.214 |
| | July 24 | 2.445 | 0.104 | 0.10 | | 0.306 |
| 27 | July 3 | 2.885 | 0.079 | | | 0.109 |
| | July 21 | 2.745 | 0.100 | 0.107 | | 0.201 |
| | August 1 | 2.585 | 0.090 | 0.079 | | 0.214 |
| 30 | August 1 | 2.280 | 0.071 | | 0.123 | |

* Struggled and micturated during collection of normal specimen.

† The ether was too concentrated (in jar); artificial respiration had to be performed.

‡ The animal had distemper and had lost weight.

§ Blood was obtained with difficulty; finally the femoral artery was cut and sample A collected into 2 per cent oxalate (without laking), and B into water (well laked).

‖ These cats were kept tied on the board during the period of frightening; the others were placed in a cage.

■ Very much excited during collection of normal specimen.

• A, collected immediately after the animal was tied on the board; B, after it had been tied for eight minutes on the board.

nephrin is not essential to the production of the hyperglycemia. The question whether these experimental hyperglycemias depend upon nervous influences exerted upon the liver so as to hasten the transformation of glycogen into dextrose, or upon a more direct influence, e.g., through changes in the hydrogen-ion concentration of the blood (7) does not concern us here. In one experiment (cat 113) after the right adrenal had been excised and the nerves of the left cut, as in the ordinary operation, the right major splanchnic was also divided after an interval of twenty-seven days. Fourteen days after the second operation, the animal being in excellent condition, the preliminary blood sample collected while the cat was quiet contained 0.089 per cent of dextrose. A second sample, taken after the animal had been frightened by a dog for thirty-eight minutes, contained 0.084 per cent. Urine passed two to three minutes after the end of the frightening period gave a negative test for sugar. A third blood sample obtained after fifteen minutes of asphyxia, immediately succeeding the period of fright, contained 0.153 per cent. The asphyxia hyperglycemia was therefore unmistakably produced in this animal after section of both splanchnics. However, our problem was merely to determine whether those hyperglycemias could or could not be produced in the absence of epinephrin. If epinephrin is not necessary for the marked and prompt augmentation of the blood-sugar observed in the conditions studied, then the deductions which have been drawn as to the importance of the epinephrin secretion of the adrenals in the mobilization of sugar can no longer be upheld.

We desire to make it clear that our present conclusions concern solely the hyperglycemia produced by asphyxia and etherization in cats. Whether such a hyperglycemia as that produced by piqûre, in which according to the best evidence the nervous system is essentially concerned, can also be elicited in the absence of epinephrin we have not as yet sufficient data to decide. The hypoglycemia described by certain observers after adrenalectomy was not seen in our animals. The average for 19 "normal" or preliminary blood samples from the control cats was 0.096 per cent. of dextrose. The average for 19 similar samples from the cats which had been subjected to the adrenal operation was the same (0.095 per cent.).

## SO-CALLED EMOTIONAL HYPERGLYCEMIA

A question which has been much discussed and which cannot very well be avoided in work on the blood-sugar, is the influence of emotional

disturbances upon the sugar content. Some writers (8) have convinced themselves that emotional hyperglycemia is so easily produced in the ordinary laboratory animals that it is impossible to obtain "normal" sugar percentages unless great precautions are adopted to prevent excitement. Others (9) have found it difficult to convince themselves that a real emotional hyperglycemia exists. We have no desire to enter into this question except insofar as it arises out of our own work. The frightening experiments were merely incidental, advantage being taken, as already mentioned, of opportunities afforded by animals which were particularly quiet during the withdrawal of the preliminary blood specimen, to see whether any marked or constant difference would be found when they were then frightened, usually by a barking dog. Since the adrenals have been supposed by some observers to be concerned also in the production of emotional hyperglycemia, we made such experiments not only on normal cats but also on cats which had undergone the adrenal operations. As will be seen from the results given in the protocols and tables, we have been unable to demonstrate in normal animals any constant increase in the percentage of sugar in the blood which could be considered as associated with emotional excitement. Further, our experiments do not reveal any essential difference between the results of emotional excitement on the blood-sugar in the normal and in the operated cats. We do not claim that our observations disprove the existence of a true emotional hyperglycemia, but they do suggest that if it exists it is a rather infrequent phenomenon, not to be elicited at will, in cats at least, as the asphyxia hyperglycemia can be elicited and so insignificant in amount that very numerous observations would be necessary to disentangle it from the uncontrollable variations in the sugar content.

The possibility must be admitted that different species of animals, perhaps different individuals of the same species, may vary in their susceptibility to emotional excitement in this regard. If this were so, man might be expected to be more susceptible than lower animals. On the other hand, great differences in the effects of such conditions as asphyxia and ether anesthesia would scarcely be looked for. However this may be, a survey of the data in the literature as well as our own data, indicates that there is a fundamental distinction between asphyxial and post-anesthetic hyperglycemias, which are well established and easily verifiable, and "emotional hyperglycemia," the existence of which is asserted by some authors on the basis of small and inconstant differences in the blood sugar content, which other writers consider to fall within the limits of variation of the normal. As regards any relation between the epinephrin discharge and emotional hyperglycemia, even granting the existence of the latter,

our experiments seem to show that there is no such association. The so-called "Fesselungs" glycosuria and hyperglycemia perhaps stand on a different footing from emotional glycosuria and hyperglycemia; for there are several factors besides the emotional one which might possibly affect the blood-sugar content of an animal tied down in an abnormal position and struggling to free itself. Such data as have been incidentally accumulated by us on this point seem to indicate a real increase in the percentage of blood-sugar when the animal has been kept for some time on the board, whether or not it has been purposely "frightened." However, we do not desire to lay stress on these observations, since where the maximum changes in the sugar content are small large numbers of experiments would be necessary to reach a safe conclusion. The three cats included in table 6 had only recently come to the laboratory. If this circumstance had anything to do with the result, we should be inclined to find the explanation rather in their nutritive condition than in greater susceptibility to excitement on account of the strangeness of their surroundings. For cats which

TABLE 6

| NUMBER OF CAT | DATE | TIME ON BOARD | DEXTROSE | REMARKS |
|---|---|---|---|---|
| | | *minutes* | *per cent* | |
| 23 | June 19 | 3 | 0.178 | Cried during puncture of vein |
| | | 20 | 0.216 | Cried during puncture of vein |
| 24 | June 19 | 8 | 0.079 | Excited |
| | | 22 | 0.135 | Excited |
| 25 | June 19 | 3 | 0.131 | Excited |
| | | 13 | 0.164 | Excited |

were to all appearance much more strongly excited, but were not kept tied down, showed no definite increase in the blood-sugar.

Observations on animals which were frightened while tied on the board are given elsewhere in the paper (table 5). Where we wished to study the effect of emotional excitement alone, the animals were set free after the preliminary specimen of blood had been obtained, and were frightened in a cage.

## IS THE OUTPUT OF EPINEPHRIN INCREASED IN NORMAL ANIMALS BY CONDITIONS WHICH CAUSE OR HAVE BEEN SUPPOSED TO CAUSE HYPERGLYCEMIA?

If the hyperglycemias studied do not depend upon epinephrin, the attempts which have been made to show that the conditions associated with the increased blood-sugar content also are associated with increased output of epinephrin would, even if they were successful, have

no bearing upon the mechanism or significance of the hyperglycemias in question. Even if it were demonstrated that asphyxia, etc., caused a marked increase in the epinephrin output, this could not be used as an argument in favor of the theory that it is a function of the adrenals to aid in the mobilization of sugar, once it was shown that this mobilization could occur all the same in the absence of epinephrin. We have, however, failed to demonstrate any definite increase in the output of epinephrin either through asphyxia or through stimulation of sensory nerves (10).

As for emotional disturbance, no method of satisfactorily testing the question has occurred to us. General anesthesia, of course, cannot be employed and the collection of the unmixed adrenal vein blood is therefore impracticable. The method adopted by Cannon and de la Paz could at most yield information as to the concentration of epinephrin in the inferior cava blood. They do not seem to have made any assay of the concentration in their published work; but the concentration alone, unless the amount of blood passing the point of collection in a given time is known, does not permit the rate of liberation of epinephrin to be calculated. Nevertheless, in order to have first-hand experience of the method we repeated the observations of Cannon and de la Paz in three cats, imitating in every particular their procedure, except that the blood specimens were tested on rabbits' intestine and uterus segments instead of cats' intestine strips. Cannon and Hoskins in their work on asphyxia and sensory stimulation used rabbits' intestine segments for the tests, and state that they are not inferior to cats' intestine strips.

In testing the bloods we did not empty the cylinder when replacing one liquid by another, as apparently Cannon and his associates do, for this produces a distortion of the curve at the critical point owing to the weight of the segment coming on the lever when the cylinder is emptied, and coming off the lever when the new liquid is introduced. A further objection to this method is that a segment which has been immersed in a liquid of given temperature saturated with oxygen is suddenly exposed to air, and then again suddenly immersed in another liquid. This can hardly be done without altering the temperature and oxygenation of the segment, and artificial effects including inhibition may sometimes be produced in this way. As in all our previous work, the contents of the cylinder were changed by displacement from below up, the new liquid, previously well oxygenated, being run in gently from a pipette drawn out to a fine point, and the old liquid overflowing into the bath. The pointed end of the pipette is bent at about a right angle so that it is easily introduced to the bottom of the cylinder without disturbing the preparation. The same quantity of liquid is always introduced when comparative observations are

being made, the pipette being filled to a mark. The amount of admixture with the liquid which is being displaced is small and approximately constant; and all that is necessary to eliminate any error due to admixture, and to make the liquid in the cylinder precisely the same as that in the stock from which it is taken, is to run in a sufficient excess of the liquid beyond the amount corresponding to the capacity of the cylinder. The curve is unaffected by the changing of the liquid in the cylinder except insofar as it produces a physiological alteration in the segment. Unless the point at which the change is made is marked on the tracing, it would be impossible to identify it. If it is desired to change the liquid by drawing off the contents of the cylinder, which would be advantageous when it is necessary to save them, this should be managed so that the new liquid enters the cylinder at the same rate as that at which the old liquid is withdrawn.

*Cat 25.* Condensed protocol. Female. Weight, 2.475 kgm. The cat was secured on a comfortable holder of the same kind as that used by Cannon for his work with the Roentgen rays on the movements of the alimentary canal. Under local anesthesia with ethyl chloride, the left femoral vein was exposed. A flexible catheter oiled inside and outside was introduced with its opening at a level 5 to 6 mm. anterior to the orifices of the adrenal veins. The level of the catheter was verified at the end of the experiment. While it could not be said that the animal showed no discomfort, the degree of disturbance during the "quiet" period was no doubt much less than when the cat was frightened by a barking dog. Specimens of blood were obtained through the catheter with the aid of an aspirator, as follows:

1. While the cat was quiet.
2. After 1½ minutes excitement by the dog.
3. After 3 minutes excitement by the dog.
4. After 13 minutes excitement by the dog. The cat micturated.
5. After 15 minutes rest while the animal was fairly quiet.

The sixth catheter specimen was obtained with the catheter inserted so that its orifice was about 8 cm. below the adrenals. This specimen was taken fifteen minutes after the fifth catheter specimen. A sample of blood was then obtained from the femoral vein through a cannula, and another sample from the carotid artery. The bloods were defibrinated and tested on rabbit intestine and uterus segments. Combined weight of adrenals, 0.38 gram.

Figures 13 to 15 show some of the tracings. Those in figure 13 afford no evidence that blood collected at the level of the adrenals during excitement caused any inhibition of the rabbit intestine segment when it displaced blood collected at the same level with the animal quiet. The bloods were tried both undiluted (observation 2) and diluted with Ringer's solution (observation 27). In figure 14, blood from the same level obtained after a period of great excitement, during which the animal micturated, was caused to displace blood obtained while the animal was quiet (observation 8). Instead of inhibition there was a further increase of tone. This was not due to the so-called "sthenic"

effect of a small concentration of epinephrin, for a similar increase of tone was seen when the "quiet" specimen replaced the "excited" specimen (observation 10). In figure 15, blood collected during excitement from the adrenal level produced also some increase of tone instead of inhibition when it replaced indifferent venous blood from the lower part of the inferior cava (observation 48). The effect was not essentially different from that caused by blood collected from the adrenal level after a period of quiet when it replaced blood previously drawn during excitement (observation 46). The result of this experiment, then, was negative, the adrenal vein blood, which doubtless contained epinephrin, being too much diluted by the general mass of epinephrin-free blood in the cava to yield reactions with the intestine segments (10), (11), (12).

Fig. 13. Intestine tracings. Bloods collected by catheter from cat 25. At *1* Ringer's solution was replaced by the first catheter specimen, from the level of the adrenals, collected while the cat was quiet, and this at *2* was replaced by the second catheter specimen collected at the same level after one and one-half minutes of excitement. Both bloods were undiluted. At *26* Ringer was replaced by the first catheter specimen (quiet) and this at *27* by the third catheter specimen, collected after three minutes of excitement. Both bloods were diluted with four volumes Ringer. (Reduced to two-thirds.)

It is surprising how little account has been taken by many writers on this subject of the great dilution which any epinephrin liberated from the adrenals must undergo before it reaches the systemic capillaries and veins, apart from loss due to its oxidation or removal. A little reflection on this point would have made the concentrations of epinephrin sometimes alleged to have been found in venous blood incredible, because they would involve incredible concentrations in the blood leaving the adrenals. In a quite recent paper, e.g., Herring (13) quotes A. Fraenkel (14) and Broking and Trendelenburg (15) as having shown that in Graves' disease the epinephrin content of the blood obtained from an arm vein is increased, as compared with the normal blood. Now Fraenkel reports that he found

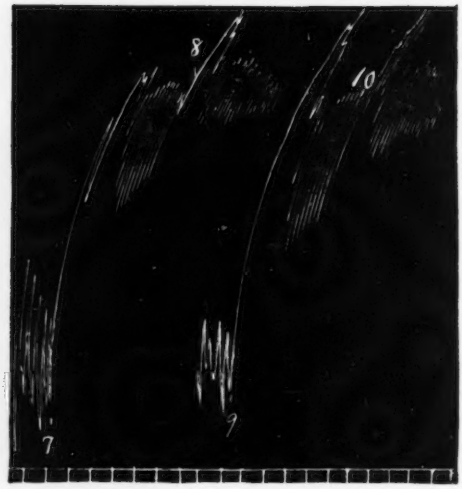


Fig. 14. Intestine tracings. Bloods from same cat used in figure 13. At *7* Ringer's solution was replaced by the first catheter specimen (quiet), and this at *8* by the fourth catheter specimen collected at the adrenal level after thirteen minutes of great excitement. At *9* Ringer was replaced by the fourth catheter specimen, and this at *10* by the first catheter specimen. (Reduced to two-thirds.)

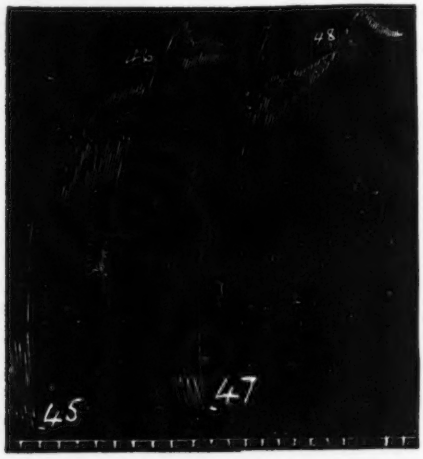


Fig. 15. Intestine tracings. Bloods from same cat as in figures 13 and 14. At *45* Ringer was replaced by the fourth catheter specimen (excitement) and this at *46* by the fifth catheter specimen (collected without excitement). At *47* Ringer was replaced by blood from the lower part of the inferior vena cava, and this at *48* by catheter blood collected at the adrenal level after ten minutes excitement. (Reduced to two-thirds.)

in a case of Graves' disease a concentration of 1: 400,000 in the venous blood. How can a statement of this sort, which according to the best available evidence would imply a concentration in the adrenal vein blood of probably at least 1: 1000 or 1: 2000 be seriously accepted? In cats under experimental conditions which are widely believed to increase the output of epinephrin, a concentration of more than 1: 1,000,000 is rarely seen in the blood coming from the adrenals. In this connection it must be remembered that although some of the blood which passes through the adrenal cortex may come into relation with the cells of the medulla before issuing from the gland, it will be safe to assume that the amount of blood which can have effective interchange with the medulla is only a fraction of the total adrenal blood flow. The concentration of epinephrin in the blood of the medullary sinusoids must therefore be higher, perhaps much higher, than that in the blood of the adrenal veins. Trendelenburg (16) has since come to the conclusion that the method of Laewen (frog perfusion), as he applied it in his earlier work, is unreliable because of the rapid development of pressor substances in shed blood, and he does not now believe that the arterial blood (in rabbits) can contain even 1: 1,000,000,000 or 1: 2,000,000,000 epinephrin. Fraenkel states that he was able to detect epinephrin (1: 20,000,000 or more) in *all* specimens of *normal* human blood! Fraenkel's data are absolutely worthless, as he ignored the fact that it was the serum and not epinephrin in it, which produced the effects on the rabbit uterus segments, which he relied upon for estimating epinephrin. When rabbit intestine segments were employed to check the results obtained on uterus segments, no epinephrin was detected in the venous blood either of normal men (17) or of patients suffering from various diseases, including Graves' disease (18).

The result of the other two experiments on emotional excitement (cats 4 and 5) was the same as in the first experiment.

*Cat 4.* Condensed protocol. Female. Weight, 2.0 kgm. The procedure was the same in cat 25. Bloods were obtained from a catheter as follows:

1. From adrenal level while cat was quiet.
2. From adrenal level after ten minutes of intense excitement (by dog).
3. With orifice of catheter withdrawn 7 to 8 cm., in lower part of cava.

Then obtained blood from the femoral artery through a cannula. As in the other experiments, the catheter was removed, cleaned and again oiled before collection of each specimen.

In this experiment some of the blood tests on the intestine segments were carried out in the usual way. Figure 16 shows that the "excited" blood did not cause any definite inhibition when it replaced "quiet" blood. For the sake of comparison, in other tests, the cylinder was emptied when a change of liquid was made (fig. 17). The apparent increase of tone in the segment at *9* is partly due to the mechanical effect of its weight coming on the lever when the Ringer's solution was removed. The same is true of the rise of the curve at *11*, when the

"quiet" blood was withdrawn. The drop in the curve at *12* when the "excited" blood was introduced is likewise a purely mechanical effect, due to floating up of the segment. There is no inhibition. At *13* the blood was removed and the weight of the segment carried up the writing point. The drop at *14*, when Ringer's solution was introduced into the cylinder, is quite decided, since not only was the segment floated up (mechanical effect), but the increase of tone was removed by the washing out of the blood by the Ringer's solution. There is no possibility of confounding the drop at *12* with a genuine inhibition, at least when the record begins with the segment initially beating in Ringer's solution, and not after blood has been applied to it, for the curve just regains the level it started with at *11*. At *14* also there is no inhibition, the segment coming back to the same length which it had before *9*.

The third experiment, on a female cat, was performed precisely like that on cat 4 and yielded a similar result.

## SUMMARY

1. The relation of the epinephrin secretion of the adrenals to experimental hyperglycemias can be investigated under much better conditions in animals whose epinephrin output has been abolished or greatly reduced by removal of one adrenal and section of the nerves of the other, than in animals deprived of both adrenals. For in the first case the animals, after recovery from the operation, remain indefinitely in good health, whereas after total adrenalectomy observations on the blood-sugar are complicated by the fact that they must be made: *a*, practically on dying animals (unless survivors in species where accessory adrenals are common are employed) and *b*, on animals suffering from the immediate effects of a major operation and anesthetization.

2. The hyperglycemia associated with asphyxia and ether anesthesia is obtained in cats which have undergone the adrenal operation described, even when no detectable residual liberation of epinephrin is present. No essential difference could be made out in this regard between these animals and control normal cats.

3. Accordingly, the mobilization of sugar, of which these experimental hyperglycemias are the expression, is not mediated through the epinephrin secretion of the adrenals.

4. Such observations as we have made on the effect of fright do not support the view that so-called emotional hyperglycemia is a constant

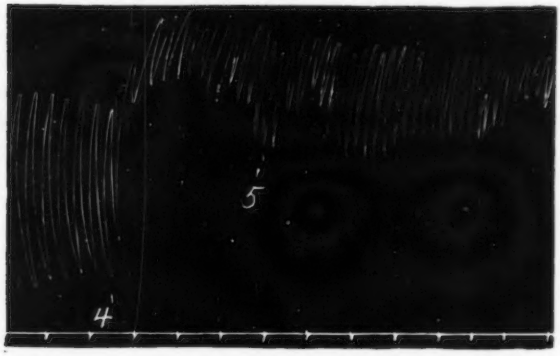


Fig. 16. Intestine tracings. Bloods from cat 4. At *4* Ringer's solution was replaced by the first catheter specimen obtained from the adrenal level while the cat was quiet. At *5*, this was replaced by the second catheter specimen from the same level after the cat had been excited for ten minutes. (Reduced to one-half.)

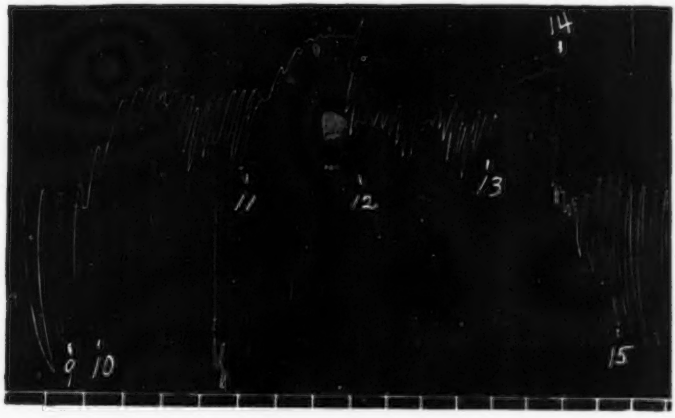


Fig. 17. Intestine tracings. Bloods from the same cat used for figure 16. At *9* the Ringer's solution was removed from the cylinder; at *10* the first catheter specimen of blood (quiet) was introduced; at *11* the blood was removed; at *12* the second catheter specimen (excitement) was introduced; at *13* the blood was removed; at *14* Ringer's solution was introduced; at *15*, the Ringer's solution in the cylinder was displaced by the introduction of Ringer's solution from a fine pipette, with its orifice at the bottom of the cylinder. (Reduced to one-half.)

or even a common phenomenon in cats. If it exists, it does not depend upon an increase in the epinephrin liberated from the adrenals. For *a*, no essential difference could be detected between the results of emotional disturbance on the blood-sugar content in the cats whose epinephrin output had been interfered with, and in the control normal cats; *b*, no evidence was obtained that emotional disturbance increases the output of epinephrin in normal cats.

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# IS DEATH FROM HIGH TEMPERATURE DUE TO THE ACCUMULATION OF ACID IN THE TISSUES?

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Reef corals from Tortugas, Florida, were kept at a constant temperature in warm ocean water for sixty minutes in a thermostat, the temperature remaining constant within about 0.1°C. throughout the hour. In this manner the temperature was found that is just sufficient to kill the coral. The results are as follows:

| | °C. |
|---|---|
| *Acropora muricata* | 34.7 |
| *Orbicella annularis* | 35.6 |
| *Porites astraeoides* | 35.8 |
| *Porites clavaria* | 36.4 |
| *Maeandra areolata* | 36.8 |
| *Porites furcata* | 36.85 |
| *Favia fragum* | 37.05 |
| *Siderastrea radians* | 38.2 |

It is apparent that those corals which live in cool, relatively agitated water free from silt are those that cannot withstand high temperatures, whereas those which live in the hot, silt-laden shallows near shore are generally speaking forms which can resist high temperature. *Favia fragum* is, however, an exception for it lives not only in the shallows close to shore but also in the pure water of the seaward reefs.

If sea water be charged to saturation with carbon dioxide from a soda-water siphon bottle filled with sea water, the corals show nearly the same relative resistance to the toxic effect of carbonic acid as they do to high temperature. Thus these corals from least to most resistant are *Acropora muricata*, *Orbicella annularis*, *P. astraeoides*, *P. clavaria*, *P. furcata*, *Maeandra areolata*, *Favia fragum* and *Siderastrea radians*; *Acropora muricata* dying in less than one hour and *S. radians* surviving for more than four hours at 28.5°C. In these experiments, however, *M. areolata* usually appeared to be slightly more resistant to $CO_2$ than did *Porites furcata*, whereas the reverse was the case with high tempera-

ture; but the two forms are so nearly alike in their reactions that this apparent difference may well be due to individual variation.

The toxic effect of $CO_2$ may, however, be independent of asphyxiation and this supposition is strengthened by the fact that with the exception of *Acropora muricata*, all of these corals can survive in the dark at 29° to 31°C. for more than eleven hours in sea water deprived of oxygen under an air pump; and even *Acropora muricata* can withstand six hours of this treatment.

It will be recalled that Henze (1) found that sea anemones consume less oxygen as the oxygen in the surrounding sea water is reduced, and McClendon (2) discovered that the medusa *Cassiopea* can live in the dark for more than eight hours without oxygen and under this condition it produces no $CO_2$. In other words, these coelenterates can, if deprived of oxygen, suspend their metabolism for a more or less protracted period.

Moreover, I find that in corals a constant high temperature causes death in the same time, and at the same degree of temperature, whether the sea water surrounding the corals has the normal concentration of about 4.3 cc. oxygen per litre, or if the oxygen be supersaturated and 6.6 cc. per litre, or reduced by being placed under an air pump to 1.7 cc. per litre. Apparently, therefore, there is no direct relation between the oxygen supply and the death temperature. Moreover, the death temperature is the same whether the corals be in sunlight or in darkness.

Using Winkler's method (3), tests were made upon five of the common reef corals of Tortugas, Florida, to determine their relative rates of consumption of oxygen. In these experiments the corals were kept in the dark to prevent photosynthesis in their commensal plant cells, for in sunlight the surrounding water soon becomes supersaturated with oxygen from this cause.

Care was also taken to use very small specimens of the various species of corals and to place them in large glass jars hermetically sealed and each containing about 2 litres of sea water. The corals were kept for five hours in these jars in darkness in a water thermostat at 28.5°C., and daily experiments were made upon each of them for eleven successive days.

The polyp-bearing area of each coral was then determined by a planimeter and the weight of living substance per square centimeter was obtained by killing specimens of these corals in formalin, hardening in alcohol and then dissolving away the calcareous substance in nitric acid and weighing the tissue in sea water.

In this manner it was found that a square centimeter of the animal substance of *Acropora muricata* weighs 0.032 grams; while *Orbicella annularis* weighs 0.17; *Favia fragum* 0.059; *Maeandra areolata* 0.109 and *Siderastrea radians* 0.125 gram per square centimeter.

The experiments showed that *Siderastrea radians* at 28.5°C. consumes 0.0032 cc. of oxygen per square centimeter of its polyp-bearing surface per hour, and as each square centimeter of the living substance of *S. radians* weighs 0.125 gram, each gram of animal substance of the coral consumes 0.0256 cc. of oxygen per hour, the oxygen being measured at 760 mm. pressure at 0°C.

Thus taking the behavior of *S. radians* as a standard and calling its oxygen consumption per gram of its living substance 1, the relative rates of oxygen consumption in the other corals at 28.5°C. are as follows:

| *Name of the coral* | *Relative oxygen consumption per hour, per gram of living substance of the coral* |
|---|---|
| *Siderastrea radians* | 1.0 |
| *Maeandra areolata* | 3.8 |
| *Favia fragum* | 5.5 |
| *Orbicella annularis* | 6.1 |
| *Acropora muricata* | 18.7 |

If the rate of consumption of oxygen be taken as a measure of the metabolism of the corals, it appears that the metabolic activity bears an inverse ratio to tke coral's ability to withstand the effects of carbonic acid or to resist high temperature. It seems possible therefore that high temperature may facilitate the accumulation of acid in the tissues and this may cause death, acids being highly toxic.

In the case of the scyphomedusa *Cassiopea*, I find that for temperatures below 37° there is no time factor,—that is to say, if the pulsating subumbrella ring deprived of sense organs and having an entrapped neurogenic contraction wave (4) be taken from sea water of about 29° and placed in water of 36°C., it at once assumes a rate characteristic of this higher temperature, and this rate is the same as if the temperature had been slowly raised so that two or more hours elapsed before the temperature rose from 29° to 36°C. Moreover, the reaction is completely reversible, the rate returning at once to the original when the ring is replaced in sea water at 29°.

At about 37° or 38°, however, the rate of the nerve impulse begins to decline sharply and I find that this is subject to a time factor, the decline becoming more and more pronounced as the heat, even though

constant in temperature, is continued. Moreover, when the highly-heated ring is replaced in sea water at 29°C., the pulsation is slower than it was before being subjected to the excessive heat. Effects such as these were described by Sachs (5) and Blackman (6) in plants and by others in animals.

After an hour or more, however, a more or less complete recovery of rate may take place, especially if the heat had not been too great or exposure to its influences too long. In other words, something toxic seems to have been caused to accumulate by the excessive heat, and this something is slowly eliminated after the animal has been returned to a normal temperature.

Thus in a typical case, a nerve wave in *Cassiopea* having a rate of 100 at 29.3° had the following rates when heated to 37.8°C.:

| *Rate* | *Temperature °C.* |
|---|---|
| 100 | 29.3 |
| 125 | 35.6 |
| 126 | 36.7 |
| 127 | 37.3 |
| 120 | 37.8 |

Thus the nerve conduction became more and more rapid up to 37.3°C. above which it declined sharply, becoming 120 after about twenty minutes at 37.8°C. The animal was then taken from 37.8°C. and replaced in sea water at 29.3°C., and its rate declined at once to 80, thus being 20 per cent less rapid than it was at the beginning of the experiment. After remaining in sea water at 29.3°C. for nearly two hours, however, the original rate was completly restored.

It will be recalled that Blackman (6) for plants, and Harvey (7) for animals advanced the theory that some enzyme may be destroyed by excessive heat, and in animals being essential to nerve conduction, its loss caused the rate to decline.

It is possible however, as shown by our experiments, that some toxic acid substance, possibly carbonic acid, accumulates under the influence of excessive heat; its rate of formation being commensurate with the metabolism of the tissues. It is easy to see how an acid of this sort might be eliminated and the rate gradually restored when the animal is replaced in normal sea water, whereas if an enzyme were destroyed it might not so readily be replaced.

In any event, one or the other of the above-mentioned hypotheses seems more in accord with the facts than does Winterstein's (8) asphyxi-

ation theory, or the theory that death from heat is due to coagulation of proteid substances.

Death occurs at too low a temperature for coagulation in most if not all proteids, and when killed, the animals are fully relaxed as shown by Harvey (7). Moreover, coagulated proteins could not readily be eliminated when the animal was restored to water at normal temperature, coagulation being practically non-reversible (9).

## SUMMARY

It seems possible that death from high temperature may be due to the accumulation of acid (possibly $H_2CO_3$) in the tissues, the rate of formation of this acid being commensurate with the rate of metabolism of the tissues. Thus animals of the same class having a high rate of metabolism, as measured by oxygen consumption, are more sensitive to heat and to $CO_2$ than are those having a low rate of metabolism.

Death at high temperature is not due to asphyxiation.

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# THE PHENOL EXCRETION OF GUINEA-PIGS MAINTAINED ON AN EXCLUSIVE OAT DIET

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Following the observation in 1907 by Holst and his pupils (1) that guinea-pigs fed on an oat or other exclusive cereal diet developed a condition which they considered identical with human scurvy, many anatomical and histological studies have confirmed repeatedly the fact that the condition may be produced at will in these animals by an exclusive cereal diet. Slight progress has been made however in the chemical interpretation of the marked changes observed, the work being confined largely to attempts to discover the substances whose presence or absence occasions the disease. The present authors have shown in a former paper (2) that the urea content of the blood and tissues of scorbutic guinea-pigs may be increased markedly above the normal and that this increase is not to be explained by partial starvation or lack of water alone.

In continuation of this previous work we have studied the hydrogen ion concentration of the urine and daily eliminations of total nitrogen, urea, ammonia, calcium and phosphorus of guinea-pigs on an exclusive oat diet. No marked changes were observed in the metabolism of these animals as compared with normal guinea-pigs (3). The hydrogen ion concentration of the urine rose but this may be explained by the partial starvation resulting from the diminished food intake. The slight variations in the eliminations of calcium and phosphorus were too irregular to admit of interpretation. These findings are similar to those recently reported by Baumann and Howard (4) in a study of the mineral metabolism in experimental scorbutus of the guinea-pig. Increased losses of calcium and phosphorus and less marked losses of other elements except magnesium were noted but the interpretation of their results, as those of all similar experiments with guinea-pigs in which there is a falling off in the consumption of food as the scorbutic

condition becomes more pronounced, is made difficult by insufficient protein intake and total calorific value of the diet, which make it impossible to estimate to what extent partial starvation was responsible for the changes in metabolism observed.

McCollum and Pitz (5) hold that the difficulty in experimental scurvy of the guinea-pig is not one of lack of "vitamines" or other accessory food substance but is caused by undue retention of the feces due to impaction of the cecum, which is large and delicate in the guinea-pig. They were able to cure guinea-pigs after the onset of marked scorbutic symptoms by the ingestion of liquid petrolatum and maintained pigs in normal condition over considerable periods of time on an oat diet provided a laxative (phenolphthalein) was also added. Impaction of the cecum may cause mechanical injury to the intestinal wall allowing access of bacteria to the tissues or the injury may be due primarily to the absorption of toxic products of bacterial metabolism from the intestine.

In view of these recent studies of McCollum and Pitz, it has seemed of value to report briefly certain experiments dealing with the phenol content of the urine of guinea-pigs on an oat diet. The phenols of the urine (6) derive their origin largely from bacterial putrefaction of proteins and amino acids in the intestine and it was believed that any marked increase in the bacterial action would be manifest by an increased phenol excretion in the urine, especially since the retention of feces should afford optimum conditions for the absorption of the products of putrefaction. On the other hand it was realized that phenols are not the only toxic products of bacterial action, that they are derived only from the putrefaction of the aromatic amino acids and are therefore not absolute indices of the intensity of bacterial processes in the intestine.

The animals were confined in the cages described elsewhere and the general methods of former experiments followed (3). In most of the experiments a fore-period, during which the animal was maintained on a carrot diet, preceded the experimental oat diet in order to furnish figures for the phenol content of normal guinea-pig urine. The guinea-pig's diet normally consists of succulent food with the water necessary for life supplied in the food. On the oat diet it had been previously observed that the animals received an insufficient supply of water and refused to drink from dishes placed in the cage. Hence in order to insure reasonably large volumes of urine water was administered daily through a pipette or medicine dropper in the amounts indicated in the tables. Oats were placed in the cage daily and the portion uneaten

removed and weighed the following day. An attempt was made to overcome the factor of partial starvation referred to by forced feeding. The oats were finely ground in a coffee mill, made into a thin paste with water and fed through a pipette. In this manner 10 to 15 grams of oats were successfully fed. The urine was collected daily under toluene, placed in the ice chest and two consecutive days' samples combined for the analyses. Phenols were determined according to the methods of Folin and Denis.

The results are given in tables 1 to 4. In no case was there evident a rise in the urinary excretion of phenol as the scorbutic condition progressed. In those experiments in which forced feeding was not attempted toward the end of the experimental period and the animal was allowed to fast, a marked drop in the excretion followed. This was presumably due to starvation since in this condition there is a diminished phenol excretion (6). No notable changes in the degree of conjugation of the phenols were apparent. If the scorbutic condition is due to an increased bacterial action and absorption of toxic products from the intestine, the phenols are not involved. It seems more probable

TABLE 1

*Guinea-pig B*

| DAYS | WEIGHT | FOOD INTAKE OATS | WATER INTAKE | URINE | PHENOLS | |
|---|---|---|---|---|---|---|
| | | | | | Total | Free |
| | *grams* | *grams* | *cc.* | *cc.* | *mgm.* | *mgm.* |
| 1– 2 | 560 | 34 | 100 | 165 | 42 | 26 |
| 3– 4 | 540 | 40 | 100 | 83 | 40 | 24 |
| 5– 6 | 528 | 35 | 105 | 76 | 38 | 20 |
| 7– 8 | 535 | 44 | 102 | 57 | 42 | 24 |
| 10–11* | 540 | 46 | 106 | 68 | 42 | 26 |
| 12–14* | 535 | 37 | 100 | 60 | 42 | 26 |
| 15–16 | 523 | 37 | 100 | 78 | 42 | 26 |
| 17–18 | 514 | 29 | 95 | 73 | 40 | 24 |
| 19–20 | 496 | 16‡ | 95 | 80 | 38 | 26 |
| 21–22 | 466 | 11 | 105 | 97 | 39 | 26 |
| 23–24 | 440 | 16 | 80 | 65 | 38 | 28 |
| 25–26 | 441 | 18 | 90 | 57 | 42 | 26 |
| 27–28 | 388 | 12 | 70 | 50 | 38 | 28 |

* A day's sample lost in each case, the 9th and 13th.

‡ Forced feeding started.

*Autopsy:* Killed. Stomach full of oats. Marked hemorrhages and enlargements of costachondral junctions. Teeth slightly loosened. Blood, urea 107 mgm. per 100 cc.

TABLE 2

*Guinea-pig J*

| DAYS | WEIGHT | FOOD INTAKE | WATER INTAKE | URINE | PHENOLS | |
|---|---|---|---|---|---|---|
| | | | | | Total | Free |
| | *grams* | *grams* | *cc.* | *cc.* | *mgm.* | *mgm.* |
| 1– 2 | 527 | 200 carrots | 0 | 155 | 40 | 25 |
| 3– 4 | 525 | 200 carrots | 0 | 110 | 40 | 24 |
| 5– 6 | 520 | 150 carrots | 0 | 95 | 40 | 28 |
| 7– 8 | 508 | 63 oats | 80 | 65 | 48 | 34 |
| 9–10 | 510 | 62 oats | 74 | 35 | 42 | 28 |
| 11–12 | 534 | 55 oats | 90 | 25 | 46 | 32 |
| 13–14 | 532 | 54 oats | 94 | 45 | 50 | 34 |
| 15–16 | 526 | 61 oats | 93 | 65 | 48 | 32 |
| 17–18 | 523 | 50 oats | 90 | 55 | 42 | 32 |
| 19–20 | 480 | 28 oats | 75 | 75 | 40 | 26 |
| 21–22 | 438 | 7 oats | 65 | 70 | 40 | 22 |
| 23–24 | 388 | 0 oats | 77 | 90 | 32 | 20 |
| 25–26 | 364 | 0 oats | 65 | 65 | 28 | 18 |

*Note:* Died in cage.

TABLE 3

*Guinea-pig K*

| DAYS | WEIGHT | FOOD INTAKE | WATER INTAKE | URINE | PHENOLS | |
|---|---|---|---|---|---|---|
| | | | | | Total | Free |
| | *grams* | *grams* | *cc.* | *cc.* | *mgm.* | *mgm.* |
| 1– 2 | 447 | 200 carrots | 0 | 155 | 42 | 28 |
| 3– 4 | 436 | 200 carrots | 0 | 135 | 42 | 28 |
| 5– 6 | 428 | 57 oats | 83 | 55 | 42 | 28 |
| 7– 8 | 431 | 33 oats | 80 | 60 | 38 | 28 |
| 9–10 | 422 | 30 oats | 84 | 60 | 40 | 28 |
| 11–12 | 425 | 41 oats | 87 | 60 | 38 | 26 |
| 13–14 | 415 | 34 oats | 80 | 65 | 38 | 28 |
| 15–16 | 396 | 31 oats | 76 | 68 | 40 | 28 |
| 17–18 | 358 | 10 oats | 76 | 80 | 34 | 24 |
| 19–20 | 312 | 1 oats | 50 | 70 | 32 | 22 |
| 21–22 | 310 | 17?* oats | 45 | 45 | 28 | 18 |
| 23 | 279 | 5?* oats | ? | 20 | | |

* Forced feeding.

*Autopsy:* Killed. Blood, urea, 91 mgm. per 100 cc. Much emaciated. Stomach and intestines full of ground oats. Marked hemorrhages around ribs and knee joints.

TABLE 4

*Guinea-pig L*

| DAYS | WEIGHT | FOOD INTAKE | WATER INTAKE | URINE | PHENOLS | |
|---|---|---|---|---|---|---|
| | | | | | Total | Free |
| | *grams* | *grams* | *cc.* | *cc.* | *mgm.* | *mgm.* |
| 1– 2 | 485 | 190 carrots | 0 | 110 | 38 | 26 |
| 3– 4 | 483 | 195 carrots | 0 | 100 | 30 | 20 |
| 5– 6 | 447 | 45 oats | 53 | 45 | 30 | 20 |
| 7– 8 | 452 | 32 oats | 64 | 25 | 32 | 22 |
| 9–10 | 446 | 35 oats | 70 | 45 | 32 | 22 |
| 11–12 | 445 | 30 oats | 66 | 35 | 32 | 22 |
| 13–14 | 422 | 23 oats | 70 | 60 | 30 | 20 |
| 15–16 | 393 | 18 oats | 44 | 40 | 30 | 20 |
| 17–18 | 365 | 7 oats | 50 | 50 | 30 | 20 |
| 19–20 | 329 | 2 oats | 56 | 68 | 20 | 12 |
| 21–22 | 307 | 0 oats | 34 | 60 | 18 | 10 |
| 23–24 | 284 | 0 oats | 32 | 38 | 16 | 10 |

*Autopsy:* Killed. Few slight hemorrhages in ribs. Small amount of fecal matter in intestine.

that the other theory suggested by McCollum may be the correct one, that the injury to the intestine is mechanical, permitting bacterial invasion of the tissues, a theory which is borne out by the bacteriological findings of Jackson and Moody (7).

## SUMMARY

In guinea-pigs on an oat diet no changes in the urinary elimination of phenols nor in the degree of conjugation of the phenols were observed provided the factor of partial starvation was ruled out. This is believed to indicate that no increased bacterial action occurs in the intestine of scorbutic guinea-pigs despite the difficulty of evacuation of the feces.

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# FORMULA FOR RATE OF NERVE CONDUCTION IN SEA WATER

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Ring-shaped strips of subumbrella tissue of the scyphomedusa *Cassiopea xamachana* were deprived of marginal sense organs and placed in concentrated sea water in order to determine the effect of concentration of electrolytes upon their rate of nerve conduction.

These results, combined with those previously obtained (1) with diluted sea water, are as follows:

TABLE 1

*Illustrated by figure 1*

| SALINITY 0/00 SALT IN GRAMS IN 1000 GRAMS OF SEA WATER | RELATIVE SALINITY, THAT OF NORMAL SEA WATER BEING 1.00 | RELATIVE ELECTRICAL CONDUCTIVITY AT 30°C., THAT OF NORMAL SEA WATER BEING 100 | HYDROGEN-ION CONCENTRATION OF THE SOLUTION IN TERMS OF $P_H$ | RELATIVE RATE OF NERVE CONDUCTION, THAT IN NORMAL SEA WATER BEING 100 | PROBABLE ERROR OF THE RATE | REMARKS |
|---|---|---|---|---|---|---|
| 18.13 | 0.5 | 54.1 | 8.1 | 54.16 | 0.79 | Normal sea water of 8.23 $P_H$ diluted with distilled water of 7.9 $P_H$. |
| 21.74 | 0.6 | 64.3 | 8.2 | 65.72 | 1.01 | |
| 25.37 | 0.7 | 71.5 | 8.2 | 73.91 | 1.16 | |
| 29.00 | 0.8 | 81.4 | 8.2 | 79.51 | 1.18 | |
| 32.61 | 0.9 | 92.2 | 8.2 | 91.44 | 1.00 | |
| 34.42 | 0.95 | 96.4 | 8.2 | 96.23 | 0.67 | |
| 36.24 | 1.00 | 100.0 | 8.22 | 100.0 | | Normal sea water of 8.2 $P_H$. |
| 39.58 | 1.092 | 107.8 | 8.23 | 101.3 | 0.52 | Sea water evaporated in sunlight and its alkalinity restored to 8.1–8.2 $P_H$ with HCl. |
| 41.59 | 1.148 | 113.6 | 8.2 | 107.5 | 0.71 | |
| 49.18 | 1.357 | 128.7 | 8.13 | 104.3 | 0.95 | |
| 60.96 | 1.682 | 153.9 | 8.1 | 76.9 | 0.63 | |

The sea water was concentrated by evaporation in sunlight, and its alkalinity restored to that of normal sea water by adding a slight amount

of hydrochloric acid, the hydrogen-ion concentration being determined colorimetrically by comparison with a graded set of thymolsulphonephthalein tubes prepared and standardized by Prof. J. F. McClendon. These readings were also checked by comparison with those of a Leeds and Northrup potentiometer.

The hydrogen-ion concentration of the sea water at Tortugas is usually between 8.1 and 8.23 $P_H$, and many experiments made in 1917 showed that the rate of nerve conduction remains practically constant and normal in sea waters ranging from 7.00 to 8.65 $P_H$; the relatively acid water being made from normal sea water treated with HCl, and the alkaline water being made from normal sea water treated with $HNaCO_3$ and $Na_2CO_3$.

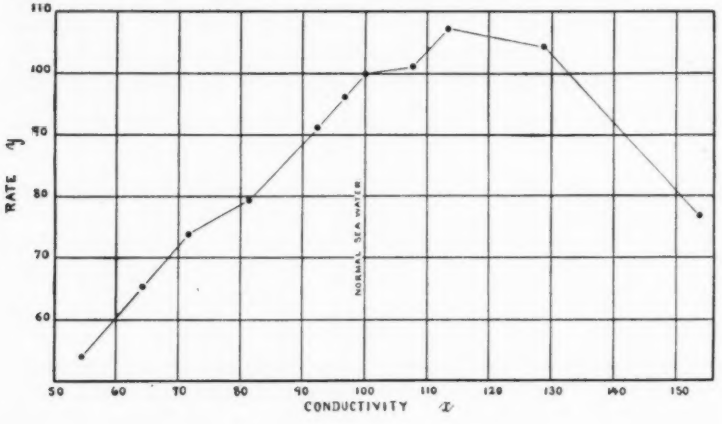


Fig. 1

If, however, the water is made as acid as 6.7 $P_H$, the rate falls slightly so as to be about 98, that in normal sea water being 100; and at 6.3 $P_H$, the rate declines to 80 with greatly reduced muscular activity; but it recovers in all respects almost immediately upon being replaced in normal sea water.

At 30°C. the neutral point is about 6.8 $P_H$ and it then appears that in normal sea water any excess of $H^\circ$ over $OH'$ is decidedly depressant while correspondingly alkaline sea water produces no ill effects.

Assuming the electrical conductivity of normal sea water of 36.24 0/00 salinity and 8.22 $P_H$ to be 100, it appears from table 1 and figure 1 that the rate of nerve conduction augments in practically a straight

line as the relative electrical conductivity of the sea water increases from 51 to about 108; the formula being $y = 0.954\ x + 4.4$ where $y$ is the rate of nerve conduction, that in natural sea water being 100; and $x$ is the electrical conductivity of the solution, that of normal sea water being 100.

Above 108, however, the rate falls off rapidly, becoming about 77 in concentrated sea water having an electrical conductivity of 1.529, and so injurious is this concentrated sea water that complete recovery does not occur within twelve hours after being replaced in natural sea water.

The electrical conductivity of the sea water was determined by Kohlrausch's method, using a tunable telephone and a Leeds and Northrup revolving drum bridge. The figures given in table 1 are for sea water at 30°C.

It was found that the electrical conductivity of sea water of 36.24 0/00 salinity and 8.22 $P_H$, at 30°C. was 4.21 times that of $\frac{N}{10}$ KCl at the same temperature. If, however, the sea water be compared with this potassium chloride solution, both at 25°C., the ratio of their electrical conductivities became as 1 to 4.179.

The salinity of the sea water was determined by titration with silver nitrate, using potassium chromate as an indicator and titrating against a sample of standard sea water from Professor Knudsen's laboratory of the International Marine Committee.

The fact that the rate of nerve conduction increases in a nearly identical ratio in comparison with the increase of electrical conductivity suggests a causal relation between the two as was pointed out by Ralph S. Lillie (2), (3); but the same ratio exists between the concentration of the dissociated cations Na, Ca, Mg and K, surrounding the nerves, and the rate of nerve conduction. Thus the rate may be dependent upon the nature and the concentration of these electrolytes rather than upon their electrical conductivity as such. Thus, in experiments made in 1917 upon *Cassiopea*, we find that if the electrical conductivity of 0.601 *m.* NaCl be taken as 1.00, that of an isotonic solution composed of 81.1 cc. of 0.601 *m.* NaCl + 14.36 of 0.39 *m.* $MgCl_2$ is 0.932; yet if the rate in the pure sodium chloride be 100 that in the NaCl + $MgCl_2$ is 115. Thus the rate has increased while the electrical conductivity has diminished.

Other solutions were also made up of chlorides and were tested by titration with $AgNO_3$, using $K_2CrO_4$ as an indicator so that the concentration of the chlorides was made the same in all the solutions and

identical with that of natural sea water. The rate of nerve conduction was determined from the study of kymograph records. The results are stated in table 2.

While the electrical conductivity of these solutions was about the same, the rates of nerve conduction in them were widely different. Thus the two phenomena appear to me not to be so intimately related as would be implied by Lillie's reference (3) (p. 167), wherein he states that his local-action theory explains "the speed of transmission of the excitation wave and the identity of its effects with electrical effects (stimulation, inhibition, etc.)."

TABLE 2

*Showing the rate of nerve conduction in Cassiopea in various solutions all isotonic each to each; the rate in natural sea water at the same temperature being 100*

| CHARACTER OF THE SOLUTION | AVERAGE RATE OF NERVE CONDUCTION | NUMBER OF OBSERVATIONS |
|---|---|---|
| Natural sea water of $P_H$ 8.1 to 8.2 | 100.0 | |
| Artificial sea water composed of 81.1 of 0.6 *m.* NaCl + 14.36 of 0.4 *m.* $MgCl_2$ + 2.84 of 0.39 *m.* $CaCl_2$ + 1.7 of 0.6 *m.* KCl + 5.5 of water. The $P_H$ of the solution was 7.5 | 97.8 | 15 |
| 93.9 of 0.614 *m.* NaCl + 2.19 of 0.39 *m.* $CaCl_2$ + 1.61 of 0.6 *m.* KCl + 2.28 of water. The $P_H$ was 7.5 | 108.7 | 13 |
| 85.31 of 0.6 *m.* NaCl + 14.69 of 0.39 *m.* $MgCl_2$. The $P_H$ was about 7 | 96.15 | 5 |
| 98 of 0.592 *m.* NaCl + 2 of 0.39 *m.* $CaCl_2$. The $P_H$ was 7.5 | 69.5 | 17 |
| 0.614 *m.* NaCl of about 7.3 $P_H$ | 55.5 | 3 |

It may be of interest to see that diluted sea water is less depressant for nerve conduction than abnormally concentrated sea water.

The injurious effects of concentrated sea water upon regeneration and growth have been studied by Loeb (4) and by Goldfarb (5), and there is a general resemblance between their curves and ours for the rate of nerve conduction excepting that for regeneration somewhat dilute sea water seems to be more favorable than is normal sea water, whereas in nerve conduction the highest rate is obtained in slightly concentrated sea water.

SUMMARY

In sea water between salinities of 18 to 40 per cent, the rate of nerve conduction in *Cassiopea* varies as a straight line in relation to the electrical conductivity, or the degree of dissociation of the cations Na̤, K̆, Mg̈ and Ca̤ surrounding the nerves.

Thus, if $y$ be the rate of nerve conduction, that in normal sea water of 36.24 per cent salinity being 100; and if $x$ be the relative electrical conductivity of the sea water that of normal sea water of 36.24 per cent salinity and 8.22 $P_H$ being 100. then

$$y = 0.945\,x + 4.4$$

In sea water of more than 40 per cent salinity, there is a decided and constantly augmenting reduction in the rate of nerve conduction.

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1917 Volume III

# THE JOURNAL

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# Bacteriological Report on Three Samples of "Bulgara Tablets"

**(Purchased in the open market)**

## Made by The Fischer Laboratories, Chicago,

August 8th, 1917

(Condensed from the original)

PURCHASED three packages of Bulgara Tablets, Hynson, Westcott and Dunning make, for bacteriological examination. All packages were cool when obtained, showing proper storage. Cultures showed a good growth of long bacilli and no gas formation. Streptococci and other bacteria were absent. The milk cultures were coagulated as reported below and showed strong acid formation. The quantitative results were as follows:

BULGARIAN BACILLI COLONIES PER GRAM OF TABLET:

| No. 2576<br>Return date: Oct. 1, 1917 | No. 2577<br>Return date: Sept. 1, 1917 | No. 2578<br>Return date: Oct. 1, 1917 |
|---|---|---|
| Plate 1.....11,720,241 | Plate 1.....4,179,728 | Plate 1.....19,560,600 |
| Plate 2.....14,324,739 | Plate 2.....7,314,524 | Plate 2.....11,953,700 |
| Plate 3.....10,417,992 | Plate 3.....7,314,524 | Plate 3.....22,820,700 |
| Average, 12,154,324 | Average, 6,269,592 | Average, 18,111,666 |
| Coagulated in 20 hrs. | Coagulated in 36 hrs. | Coagulated in 20 hrs. |

The decreased number of organisms and the longer coagulation time found in specimen 2577 was undoubtedly due to the greater age of the package, as indicated by the return time. No matter how carefully kept, all organisms will gradually lose their virility and there will always be a gradual dying out of the bacteria.

However, the number of organisms present in even the oldest of these specimens is enormous, and taking into consideration the fact that the average reproducing time of bacteria, when placed in a growing environment, is twenty minutes—that is, the number is doubled in this time—we believe there is no question as to the therapeutic activity of Bulgara Tablets when handled as recommended within the time specified on package. From the reactions in the culture media and the microscopical characteristics of the organisms, we consider the tablets to consist of pure cultures of Bacillus Bulgaricus, Type A. We might add that after analyses of other commercial preparations of Bulgarian Bacilli we were skeptical as to the viability and virility of stock cultures of these organisms. The results of our examinations of these samples are in marked contrast to our previous experiences.

(Signed) THE FISCHER LABORATORIES,
Per C. E. M. FISCHER.

DEC 24 1917

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